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Original Articles

ACTIVATION OF ULTRAFILTRATES OF RAGWEED POLLEN EXTRACTS BY MEANS OF COLLOIDAL SUBSTANCES*†

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THE fact that ultrafiltrates of pollen extracts are less active than the extracts from which they are derived is well known. However, Benjamins, von Dishoeck, and German[1] working with ultrafiltrates of *Festuca rubra* (Red Fescue Grass), of *Dactylis glomerata* (Orchard Grass), and of *Holcus lanatus* showed that although the activity of these pollen extracts was less in the ultrafiltrates, it could be increased greatly by the addition of colloid substances to the ultrafiltrates, such as proteins, proteids, and polysaccharides. Because of the possibility that this observation may throw some light on the nature of the active substance of pollen, it seemed important to repeat this work by using ragweed pollen.

Pollen of *Ambrosia trifida* was extracted with ether and hot alcohol. One cubic centimeter of this purified pollen was extracted at room temperature with 100 c.c. of Coca's solution for several hours and ultrafiltered at a pressure of 60 pounds through discs of Nos. 1200 and 600 cellophane membranes under sterile conditions.

Intracutaneous testing on the back of a ragweed-sensitive individual was done with increasing dilutions of pollen extract, ultrafiltrate, and of ultrafiltrate of these extracts to which had been added egg albumen or casein, so that the dilution of the added protein was 1:1000 in all tests.

Table I-A shows the great decrease in activity of the pollen brought about by ultrafiltration.

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†Read before the Fifteenth Annual Meeting of the Association for the Study of Allergy, Atlantic City, N. J., June 7, 1937.

TABLE I-A

COMPARISON BETWEEN WHOLE EXTRACT AND ULTRAFILTRATE PORTION

EXTRACT				ULTRAFILTRATE			
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	ESTIMATION OF INTENSITY OF REACTION	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	ESTIMATION OF INTENSITY OF REACTION
$\frac{1}{100,000}$							
$\frac{1}{1\ M}$	10	30P	++++				
$\frac{1}{10\ M}$	8	25	++++	$\frac{1}{10,000}$	6	18	+++
$\frac{1}{100\ M}$	6	20	++++	$\frac{1}{100,000}$	3	0	+
$\frac{1}{1\ B}$	5	10	+++				
$\frac{1}{10\ B}$	5	10	+++				

TABLE I-B

EXTRACT				ULTRAFILTRATE			
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	ESTIMATION OF INTENSITY OF REACTION	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	ESTIMATION OF INTENSITY OF REACTION
$\frac{1}{100,000}$	8	35	++++	$\frac{1}{1000}$	6	25	+++
$\frac{1}{1\ M}$	5	25	++++	$\frac{1}{10,000}$	4	20	+++
$\frac{1}{10\ M}$	8	25	++++	$\frac{1}{100,000}$	3	5	
$\frac{1}{100\ M}$	5	30	+++	$\frac{1}{1\ M}$	3	5	+
$\frac{1}{1\ B}$	5	20	+++				

Dilutions range from one to one thousand, one to one million ($\frac{1}{M}$), and one to ten billion ($\frac{1}{10\ B}$). Results are recorded as the diameter of a wheal in millimeters, followed by the diameter of the erythema and whether or not there are pseudopods (p).

TABLE II

PART OF THE TOTAL NITROGEN IS REMOVED BY ULTRAFILTRATION	
Total nitrogen of extract	10.0 mg.
Total nitrogen of ultrafiltrate	9.0 mg.
Total nitrogen of residue	70.0 mg.

More than one ultrafiltration does not remove more activity than one ultrafiltration, as shown by Table III.

TABLE III

COMPARISON OF EFFECTS OF ONE ULTRAFILTRATION WITH TWO ULTRAFILTRATIONS

DILUTION	ONE ULTRAFILTRATION		TWO ULTRAFILTRATIONS	
	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/1000	8 —	40	6 —	35
1/10,000	8 —	35	7 —	35
1/100,000	6 —	35	7 —	30
1/1 M	4 —	10	2 —	4

The result recorded in Table III is to be expected, as a molecule small enough to pass through the pores of a filter once should be able to do so any number of times. This assumes that the molecule has not been adsorbed by some added substance nor has formed larger molecular or colloidal aggregates with similar molecules.

TABLE IV

COMPARISON OF AMOUNT OF ACTIVITY REMOVED BY DIFFERENT MEMBRANES

DILUTION	FILTRATE FROM MEMBRANE NO. 600		FILTRATE FROM MEMBRANE NO. 1200		NO. 600 + ALBUMIN		NO. 1200 + ALBUMIN	
	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/1000	10P —	25	7 —	20	7 —	30	7 —	35
1/10,000	6 —	25	3 —	10	7 —	25	6 —	15
1/100,000	5 —	15	3 —	5	5 —	13	3 —	8
1/1 M	3 —	5	3 —	5	3 —	10	3 —	5
1/10 M	3 —	7	3 —	5	3 —	5	3 —	5
1/100 M	3 —	7	3 —	5	3 —	5	3 —	5
A 1/1000	4 —	10						

A = albumin.

We next compared the relative efficacy of No. 1200 against No. 600 membranes and found that the No. 1200 membranes removed more activity, and that the ultrafiltrates from the No. 600 membranes could be activated to a greater degree. This is shown in Table IV.

TABLE V

COMPARATIVE EFFECTS OF ULTRAFILTRATE OF EXTRACT WITH THAT OF EXTRACT AND EGG ALBUMEN

ULTRAFILTRATE OF EXTRACT			ULTRAFILTRATE OF ALBUMIN + EXTRACT		
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	
1/1000	8	40	6	—	20
1/10,000	8	35	3	—	10
1/100,000	6	35	2	—	0
1/1 M	4	10	2	—	0

The addition of egg albumen to the ultrafiltrate and refiltration causes still further decreases of the activity in the ultrafiltrates. This is because some of the unadsorbed activity, which formerly could pass through the ultrafilter, now was adsorbed by the albumin and the resulting molecular aggregate too was larger than the filter pore. This is shown in Table V.

TABLE VI

ACTIVITY OF THE FILTRATE OF THE ULTRAFILTRATE + TRYPSIN

	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
Ultrafiltrate of extract + trypsin	1/1000	7 —	25
	1/10,000	3 —	5
	1/100,000	3 —	5
	1/1 M	3 —	5

Trypsin adsorbs the activity of the ultrafiltrate. This is before digestion, the trypsin merely acting as the albumin in the previous experiment (Table VI).

It is seen that zinc hydroxide adsorbs most of the activity of the pollen extracts. This activity is released by acid potassium phosphate.

The addition of albumin or casein greatly increases the activity of the ultrafiltrates.

Increasing the amount of protein beyond a certain point does not further increase the activity of the ultrafiltrate (Table X).

TABLE VII

COMPARISON OF ACTIVITY IN ORIGINAL EXTRACTS AND IN EXTRACTS TO WHICH ALBUMIN HAS BEEN ADDED

The addition of albumin to the original extract does not increase its activity.

EXTRACT			EXTRACT + ALBUMIN		
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/10 M	7 —	35	1/10 M	7 —	40
1/100 M	7 —	35	1/100 M	5 —	40
1/1 B	7 —	30	1/1 B	7 —	35
1/10 B	5 —	30	1/10 B	7 —	35

TABLE VIII

EFFECTS OF THE ULTRAFILTRATE TO WHICH ZINC HYDROXIDE HAS BEEN ADDED AND REMOVED

EXTRACT			EXTRACT + Zn HYDROXIDE			EXTRACT + Zn HYDROXIDE + POTASSIUM PHOSPHATE		
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL		DIAMETER OF ERYTHEMA
1/1 M	10 —	40	1/1000	10 P —	45	20 P —		55
1/10 M	10 —	40	1/10,000	7 —	35	12 P —		50
1/100 M	7 —	30	1/100,000	7 —	30	10 P —		50
1/1 B	7 —	30	1/1 M	5 —	25	7 —		45

Heating the aqueous extract at 100° C. in sealed ampoules at a pH of 7 for one hour, partially destroys its activity. Ultrafiltration still further decreases the activity of the heated extract. The addition of albumin to the heated extract seems to decrease its activity, and the addition of albumin to the heated ultrafiltrate did not produce an increase in activity.

Heated ultrafiltrates were less active than ultrafiltrates.

TABLE IX-A

EFFECT OF ADDING ALBUMIN OR CASEIN TO ULTRAFILTRATES

EXTRACT				ULTRAFILTRATES			
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	ESTIMATION OF INTENSITY OF REACTION	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	ESTIMATION OF INTENSITY OF REACTION
1/100,000	8	35	++++	1/1000	6	35	++++
1/1 M	5	25	++++	1/10,000	4	20	+++
1/10 M	8	25	++++	1/100,000	3	5	+
1/100 M	5	30	+++	1/1 M	3	5	+
1/1 B	5	20	+++	*A*			
				1/1000	4 2	15 20	++
				C			
				1/1000	3 2	10 25	++

DILUTION		DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	ESTIMATION OF INTENSITY OF REACTION
U 1/10,000	+ A	1/1000	7	40	++++
U 1/100,000	+ A	1/1000	7	40	++++
U 1/10,000	+ C	1/1000	5	33	++++
U 1/100,000	+ C	1/1000	5	25	++++

A = albumin U = ultrafiltrate C = casein

TABLE IX-B

EFFECT OF ADDING ALBUMIN TO ULTRAFILTRATES

ULTRAFILTRATE			ULTRAFILTRATE + ALBUMIN	
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/1000	6	25	8	40P
1/10,000	4	40	6	30
1/100,000	2	12	4	20
1/1 M	0	10	0	5
A 1/1000	3	0		

A = albumin

TABLE IX-C

EFFECT OF ADDING ALBUMIN TO ULTRAFILTRATES

EXTRACT			ULTRAFILTRATE			ULTRAFILTRATE + ALBUMIN	
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/1 M	10 —	40	1/1000	8 —	30	5 —	30
1/10 M	10 —	40	1/10,000	5 —	25	8 —	50
A = 3 – 0							
1/100 M	7 —	30	1/100,000	5 —	15	9 —	40
1/1 B	7 —	30	1/1 M	7 —	20	9 —	40

A = albumin

TABLE IX-D

EFFECT OF ADDING ALBUMIN TO ULTRAFILTRATES

ULTRAFILTRATE			ULTRAFILTRATE + ALBUMIN		
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/100,000	3 —	20	1/100,000	8 —	40
1/1 M	2 —	5	1/1 M	10 —	40

ULTRAFILTRATE			
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	ESTIMATION OF INTENSITY OF REACTION
1/1000	5 —	20	++
1/10,000	3 —	10	+
1/100,000	2 —	5	

Albumin = 2–20

Passive transfer tests were then done. A comparison of the ability of extract and ultrafiltrates to exhaust the site of transfer was then made.

Ultrafiltrates in a dilution of 1:1000 were about as successful as extracts in a dilution of 1:10 M in desensitizing the sites.

DISCUSSION

Skin reactions, qualitatively the same as those of pollen extracts, are obtained from ultrafiltrates of these extracts. This suggests that some

TABLE X

EFFECT OF INCREASING AMOUNTS OF ALBUMIN UPON ACTIVITY OF ULTRAFILTRATES

ULTRAFILTRATE			DILUTION $+A\frac{1}{1000}$		DILUTION $+A\frac{1}{10,000}$		DILUTION $+\frac{A}{100,000}$	
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1:1000	5	20	6	40	6	25	6	30
1:10,000	3	10						
1:100,000	2	5						
$+C\frac{1}{1000}$	4	20						
$+C\frac{1}{10,000}$	5	15						

A = albumin
C = casein

of the active substance exists in an unadsorbed state in aqueous solutions. This active principle can be almost completely adsorbed by the addition of albumin, gelatin, casein, or trypsin to the extracts. It is also partially adsorbed by zinc hydroxide and released from the zinc by acid potassium phosphate.

Ultrafiltrates can exhaust passive transfer sites of reactive serum, although greater concentrations of ultrafiltrates than of extract are needed to do this. This constitutes evidence that the active substance of ultrafiltrates is identical with that in extracts.

All tests for protein were negative in the ultrafiltrates. This is to be expected as the clinical tests for protein are far from as delicate as the skin tests for pollen activity. They do suggest, however, that if the active substance is a protein, it is of small molecular weight. Further work as to the exact pH at which adsorption to various colloids occurs should be done.

Why the protein of the skin does not perform the function of the added albumin or casein is unknown. The activation of the ultrafiltrates occurs almost instantaneously. The function of the nonspecific colloidal substance would seem to be one of supplying an adequate surface from which the pollen principle could act. If this were so, the structures of the skin ought to furnish the same mechanism, and yet no such activation takes place. Nor does the intensity of the reaction in the injected sites increase, thus ruling out a delayed reaction. Injection of ultrafiltrates into slightly traumatized tissue should be tried.

CONCLUSIONS

1. Ultrafiltrates of pollen extracts are very much less active than the extracts themselves.

TABLE XI-A

EFFECT OF HEATING UPON EXTRACTS AND ULTRAFILTRATES AND THEIR ABILITY TO BE ACTIVATED BY ALBUMIN

	HEATED EXTRACT		HEATED EXTRACT + ALBUMIN				HEATED FILTRATE + ALBUMIN				HEATED FILTRATE	
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/100	15 —	40	18 —	45	12 —	30	5 —	20	8 —	20		
1/1000	8 —	30	5 —	20	5 —	8	4 —	10	4 —	15	12 —	35
1/10,000	8 —	30	5 —	10	3 —	5	4 —	0	3 —	10	5 —	25
1/100,000	5 —	20	2 —	5	3 —	0	4 —	2	3 —	6	3 —	0
1/1 M	4 —	15	0 —	5	3 —	0	3 —	0	2 —	0		
1/10 M	4 —	10	5 —	0	3 —	0	3 —	10	2 —	0		

TABLE XI-B

EFFECT OF HEATING UPON EXTRACTS AND ULTRAFILTRATES AND THEIR ABILITY TO BE ACTIVATED BY THEM

DILUTION	EXTRACT		EXTRACT (HEATED)		EXTRACT (HEATED) + ALBUMIN		ULTRAFILTRATE		ULTRAFILTRATE (HEATED)	
	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/1000			9 P	35	7	35	8	35	8	12
1/10,000			9 P	35	7	30	7	20	3	0
1/100,000			7	18	5	20	3	0	3	0
1/1 M	8	40	3	10	5	8	3	0	3	0
1/10 M	7	25								
1/100 M	7	25								
1/1 B	3	10								
1/10 B										

TABLE XI-C

EFFECT OF HEATING UPON EXTRACTS AND ULTRAFILTRATES AND THEIR ABILITY TO BE ACTIVATED BY ALBUMIN

	EXTRACT		EXTRACT (HEATED)		EXTRACT (HEATED) + ALBUMIN		ULTRAFILTRATE		ULTRAFILTRATE (HEATED)		ULTRAFILTRATE + ALBUMIN	
DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1/1000			19 P	60	25 P	70	8	30	8	35	5	30
1/10,000			20 P	60	15 P	60	5	25	5	20	8	50
1/100,000			15 P	55	10 P	45	5	15	5	5	9	40
1/1 M	10	40	10	45	7	55	7	20	3	0	9	40
1/10 M	10	40										
1/100 M	7	30										
1/1 B	7	30										
1/10 B												

Table XII

Intracutaneous Reactions Obtained in Passive Transfer Sites

	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA	DILUTION	DILUTION	DIAMETER OF WHEAL	DIAMETER OF ERYTHEMA
1	1/1 M Ex.	10 —	35	1/1 M Ex.	8 —	10	1/1000 U.	1/10,000 Ex.	9 —	30
2	1/1 M Ex.	10 —	35	1/1000 U.	8 —	10	1/1000 U.	1/100,000 Ex.	8 —	15
3	1/1 M Ex.	8 —	35	1/1000 U.	5 —	8	1/100 M Ex.	1/1000 U.	5 —	18
4	1/1 M Ex.	10 —	35	1/1 M Ex.	5 —	8	1/1 B Ex.	1/1000 U.	4 —	5
5	1/10 M Ex.	8 —	30	1/10 M Ex.	8 —	0	1/1 M Ex.	1/1000 U.	4 —	5
6	1/10 M Ex.	8 —	25	1/1 M Ex.	7 —	0	1/1 M Ex.	1/100,000 Ex.	8 —	20
7	1/10 M Ex.	8 —	30	1/1000 U.	7 —	0	1/10,000 Ex.	1/1000 Ex.	9 —	45
8	1/10 M Ex.	6 —	25	1/1000 Ex.	10 P —	40	1/100,000 Ex.	1/1000 Ex.	9 —	40
9	1/1000 U.	7 —	25	1/1000 U.	8 —	0	1/1000 U.	1/1000 U.	3 —	10
10	1/1000 U.	7 —	25	1/1 M Ex.	8 —	0	1/1000 U.	1/1000 U.	3 —	10
11	1/1000 U.	7 —	29	1/1000 Ex.	8 P —	25	1/1000 U.	1/1 M Ex.	5 —	18
12	1/1000 U.	6 —	18	1/1000 U.	8 —	0	1/1000 U.	1/100,000 Ex.	7 —	20

Ex. = extract
U. = ultrafiltrate

2. The active substance can be adsorbed on protein or zinc hydroxide.

3. The active substance can pass through a No. 1200 membrane filter as well as a No. 600, although to a lesser degree. Activation of the No. 1200 membrane ultrafiltrates is less than that produced by ultrafiltrates of the coarser filter.

4. Activation of ultrafiltrates is brought about by proteins.

5. Heat partially destroys the activity of ultrafiltrates and extracts.

6. Ultrafiltrates desensitized sites which have been sensitized with pollen extract but to a lesser degree than the extracts themselves.

REFERENCE

1. Benjamins, C. E., V. Dishoeck, H. A. E., and German, J. L. M.: Studies on the Active Substance of Grass Pollen, J. ALLERGY 6: 335, 1935.

DISCUSSION

DR. J. A. RUDOLPH, Cleveland.—I think this paper proves one thing, that the extracts based upon nitrogen determination standardization leave much to be desired. The Doctor has pointed out that the nitrogen does not seem to differ very much in the ultrafiltrate and in the nonfiltrate extract; that if we are to depend upon nitrogen determinations as a standard for skin reactions, we are inclined to be somewhat misled. That is the one point I believe this paper does bring out, that the nitrogen determinations were very similar in both of the extracts and that in the ultrafiltered extract the reactivity by skin tests seemed to be less than the unfiltered one. This is the point I thought worth mentioning in this paper, since the results are otherwise negative.

DR. WARREN T. VAUGHAN, Richmond.—The observations of Walzer show that whatever the allergen may be it gets into the blood in a condition nearly enough like its original state so that it can be demonstrated by biologic testing of the skin even in the normal individual. This is not in accord with current teaching on the physiology of protein digestion.

Very recently an article by Chevalier in France showed that if patients who were allergic to milk were skin tested with milk, which had been partially digested in the stomach and recovered by lavage, then ultrafiltered and neutralized, positive skin reactions resulted. They succeeded in giving relief by desensitization with this so-called protein-free ultrafiltrate. This raises the question whether haptens may play a part even in protein allergy.

I would not be at all surprised to see within the next few years much more attention paid to haptens than to proteins. It is just an interesting thought. I think there are evidences of a trend in this direction. Is there a hapten factor, even in allergy?

DR. ARTHUR F. COCA, Pearl River, N. Y.—As I understood this paper, it seemed that the outstanding conclusion is that the excitant is a small molecular substance. I should not like to let that conclusion go by without some challenge, largely on technical grounds.

The cellophane membrane is not the best membrane for filtration. It is a very good membrane for dialysis when you do not care if you lose something. That No. 600 membrane, in my experience (it may be

that it is being made better now) when I was using it two years ago, leaked and I could not use it for that reason and had to use the 1200 for dialysis in order not to lose too much. I think, to be sure of that point, that you ought to use a collodion membrane which you make yourself and that it should be tested for its porosity to be sure that it will not let protein through.

The speaker was quite right in pointing out that the chemical identification or detection of protein in such low concentration is impossible and that, on the other hand, the reactions in the skin will demonstrate a very weak solution of the protein.

As to the heating experiments, I would point out that the bicarbonate solution—that was the one that was used, was it not?

DR. LONG.—For those, they were heated at a pH of 7 in sealed ampoules.

DR. COCA.—You did not use the bicarbonate?

DR. LONG.—No; I did not. I extracted with pure water.

DR. COCA.—The experiments we carried out a number of years ago on that point indicated that if the heating is done in just that way, there is no detectible loss at temperatures up to 70° at any rate.

I think that you should have considerable hesitancy, more than was brought out in your paper, in saying that the excitant in pollen extracts is a small molecular substance.

DR. VAUGHAN.—Of course, the problem as to when protein is present in sufficient quantity to be of significance is a problem that must be raised in discussions of this phase of allergy, yet we are undoubtedly getting down, if I may use the expression, to a tremendously small amount of protein.

In the early days of the study of anaphylaxis and of allergy, the most startling phenomena were observed in the study of protein anaphylaxis. Then the study spread into drug allergy and there we had to add the concept of haptens to reconcile the apparent lack of a protein factor. When we get to contact allergy, assuming it is based on the same phenomenon, we are getting away entirely from proteins.

At about the same time, parallel with these developments in the study of allergy, we find the recognition of the specific soluble carbohydrate fraction of the pneumococcus, which appears to act as a hapten. Then Grove and Coca, if I quote Dr. Coca correctly, felt that the active agent of the ragweed pollen was possibly not protein.

DR. COCA.—It had not been proved.

DR. LONG.—It seems to me that the significance of this paper is not any information which it gives us about the nature of the substance or the size of the molecule, but that these relatively active filtrates can be tremendously activated by colloidal substances. It seems to me if we could only get a little more information as to that one point, we would further our knowledge on the nature of atopens.

THE SPECIFICITY OF FUNGOUS ALLERGY*

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IN 1932, Cobe[1] of Philadelphia reported a case of a greenhouse worker who was sensitive to *Cladosporium fulvum*. This man was free of trouble for most of the year but had severe asthma as soon as the mold appeared on the leaves of the tomato plant in greenhouse cultivation, and this asthma lasted until the plants were uprooted in the latter part of August. An extract of the mold culture was made and to it the patient gave a "4-plus" skin test while control tests with other fungous extracts as well as with an extract of a normal tomato leaf gave no response.

In January, 1937, Feinberg[2] reported to one of us that he had been working on the identical problem of hay fever and asthma among tomato greenhouse workers.

In April, 1936, one of us (Guba[3]) published in the *Florists Review* a short account of the symptoms sustained by a greenhouse worker sensitive to *Cladosporium fulvum Cke.* whenever he was exposed to the mold.

In November, 1935, a patient was referred to one of us by Dr. William H. Weston, Jr., Professor of Cryptogamic Botany at Harvard. He was plant pathologist to an agricultural experimental station and observed that whenever he entered the greenhouse where tomatoes were grown, he could tell at once whether or not the plants were infected with *Cladosporium* leaf mold by the presence or absence of the immediate development of sneezing and coryza which soon led to asthma. This patient gave a marked skin test by both scratch and intradermal methods to an extract made from the growth of *Cladosporium fulvum Cke.*, but he showed no reaction to control tests with other molds or with other common allergens. During that winter, treatment with the extract of the *Cladosporium fulvum* resulted in such improvement that in the spring he was able to endure exposure to the fungus without symptoms.

More recently, it occurred to us that a study of the specificity of this particular mold extract might be important. The genus *Cladosporium* contains a large number of species some of which grow with considerable variation on Sabouraud's and other culture media. In other instances

*From the Harvard Medical School, Boston, Mass., and the Allergy Laboratory, Massachusetts General Hospital, Boston, Mass. Aided by the Eleanor Husted Leal Fund.

the variations are obscure or lacking so that cultural characteristics are not always wholly reliable in the separation of species. For this and others reasons, it seemed worth while to profit by the opportunity and test the patient with various species of *Cladosporium* by skin tests with extracts made at the same time from pure cultures.

Extracts of all molds were prepared as follows: The cultures were grown upon potato dextrose agar media in 250 c.c. Erlenmeyer flasks. After maximum sporolation had occurred, the growth was first moistened with about 5 c.c. of ether and then allowed to extract in 20 c.c. of Coca's

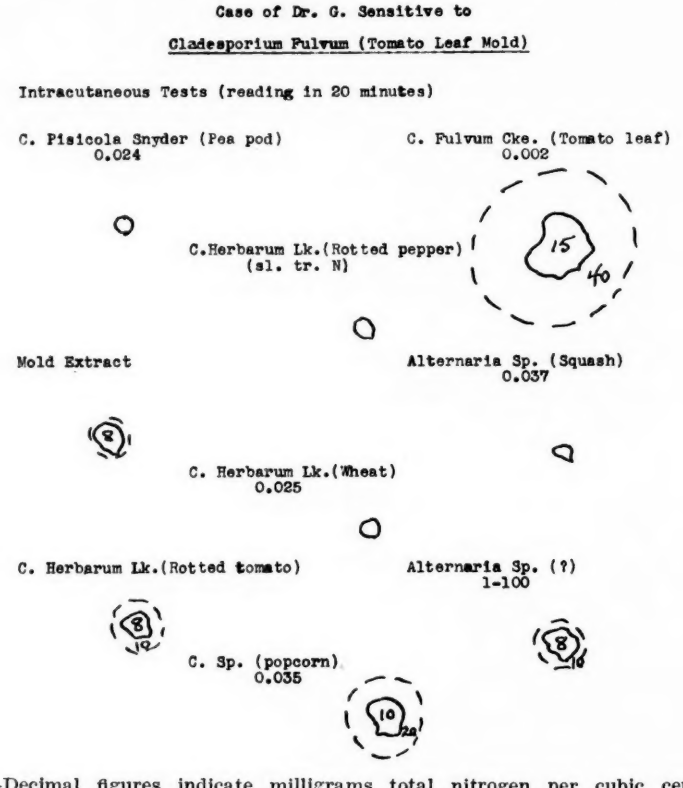

Fig. 1.—Decimal figures indicate milligrams total nitrogen per cubic centimeter. Figures within wheals indicate maximum diameters in millimeters.

buffered phosphate fluid solution for twenty-four hours. The fluid was then decanted and filtered for sterility through a Seitz filter. The total nitrogen content was determined.

The results of this first experiment are shown in Fig. 1. The large reaction to *Cladosporium fulvum* is in obvious contrast to the smaller reactions to the other molds. Furthermore, it should be noted that the test with *Cladosporium fulvum* was made with a higher dilution than the test with the other molds. A little later, it was shown that the serum of the patient was capable of transferring reagins to the arm of a normal

person. These observations seemed to be of considerable importance, both botanically and immunologically, and have been submitted along with mycological matter of allied interest for publication.*

More recently, however, we have found three similar cases in a family of greenhouse tomato growers which were studied through the courtesy of Dr. Joseph H. Dunn of Rockland, Mass. Each of the three, two brothers and their mother, was tested intradermally with extracts of various *Cladosporia*. In each case, extracts of *Cladosporium fulvum Cke.* gave large intradermal skin reactions even when used in high dilutions, whereas extracts of other species of *Cladosporium* similarly prepared

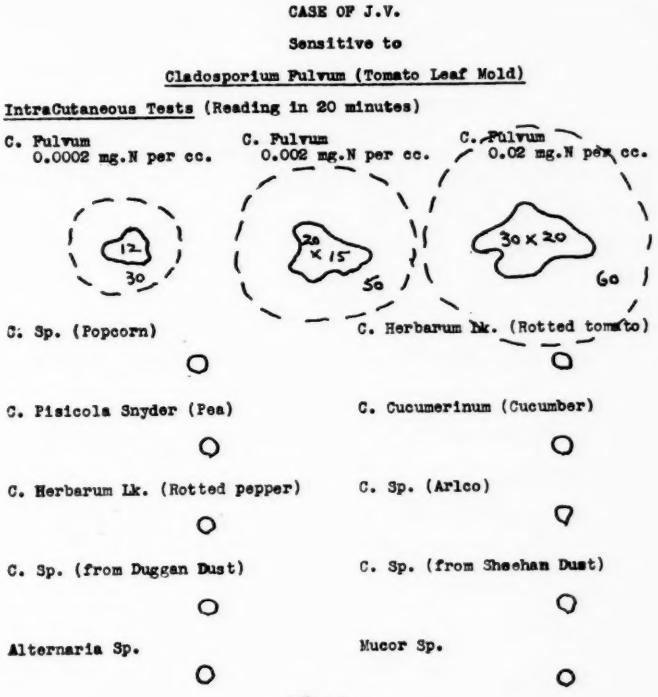

Fig. 2.

showed no appreciable skin response. In each case, positive passive transfer reactions were obtained.

The histories of these cases may prove of more interest if we first summarized the story of tomato culture in this particular greenhouse establishment. The family began raising tomatoes in 1926, having cultivated radishes and cucumbers for six years previously. One greenhouse planting is made here around August 1 of each year. As the plants increase in size, their leaves support an increasing growth of the fungus which at first appears as thin patches of white mycelial mold upon the under surface of the leaves. Later, the patches of mold turn brown in color

*Species of *Cladosporium* on Tomato and the Allergic Response in Man as an Aid to their Identification, by Emil F. Guba, Ph.D., and Francis M. Rackemann, M.D., Mycologia. In Press.

and ultimately involve the entire leaf. In this latter stage, any slight disturbances to the leaves by air currents or workers cause the release of clouds of spores into the greenhouse atmosphere. In working among the plants, the clothing of the workers readily becomes contaminated with spores of the fungus. "Dust" from the mold growth from the late summer tomato planting is common after October 1 and then increases in amount commensurate with an increase in the prevalence of the fungus until the vines are destroyed in late December. A second planting of tomatoes is made in January or February and finished off late in July. This planting usually becomes infected with the fungus early in May, and the amount of the fungus increases with warmer weather.

CASE 1.—H. V., aged forty-one years, developed typical hay fever symptoms at the age of four years. Later he received timothy and ragweed treatment with excellent therapeutic results. He never had asthma, however, until the first crop of tomatoes was raised in 1926. From that time on, he continued to have severe asthma while working among the infected vines but under no other circumstances. Eventually, his sensitivity increased to the point where he was not able even to assist in packing the picked tomatoes outside the greenhouse. In December, 1935, he ceased working with tomatoes and aside from mild wheezing when handling dry hay has had no asthma since.

CASE 2.—A brother, J. V., aged thirty-nine years, had had symptoms suggestive of allergic rhinitis since childhood but no asthma until the fall of 1926, when he first came in contact with infected tomatoes. His asthma was limited to the seasons from October to January 1, and from May to July, corresponding exactly to the growth of the fungus upon the tomato plants. He too found it necessary to leave the greenhouse in 1935, and has had no asthma since. The skin tests in his case are illustrated in Fig. 2.

CASE 3.—The mother of the two men, aged sixty-four years, had lived in her present home for twenty-nine years. In the fall of 1926, she developed attacks of sneezing with mild wheezing. Such symptoms recurred yearly from mid-October through the winter holidays. She first developed severe asthma in July of 1931, and this recurred the following fall. At that time, she was seen by one of us (Rackemann). She had dyspnea on exertion, a large heart, a blood pressure of 160/90 mm. Hg., râles in her bases, and all skin tests were flatly negative. A diagnosis of intrinsic (cardiac) asthma was made. The history in relation to her husband's and boys' occupation was missed entirely. It was sometime after that she observed her asthma was initiated or became worse when her husband returned home from working in the greenhouse. She was much better when her men changed their clothes in the woodshed.

Meantime, it is interesting that a third son, N. V., aged thirty-two years, has worked continually in the same greenhouse since 1926, but has never had any symptoms of allergy. In his case, intradermal skin tests with the same extracts were entirely negative.

DISCUSSION

These findings are of considerable importance. First and foremost, they indicate that there is a high degree of specificity among the species of the genus *Cladosporium*.

Second, if this specificity can be shown to exist in other mold groups, it may help to explain the wide divergence in the incidence of positive skin tests to mold extracts, one investigator finding them in less than one per cent and another in over fifty per cent of the patients tested.

Third, the results point to the need of study, not of molds in general, but of particular species of molds to which a particular patient may be exposed.

Patients who have asthma in certain places and who are relieved in other places and give positive skin tests to house dust, kapok, or feathers may owe their troubles to particular molds growing in the "bad" places. In the past, our attention has been focused largely upon the mold spores occurring in the outside air rather than the mold flora within dwellings. It is also true that many reported cases of inhalant mold allergy have been based upon the finding of positive skin reactions rather than a demonstration of the relationship of symptoms with the actual inhalation of the suspected mold material. The actual number of cases where specific molds have been identified and proved by provocative tests is not large.

The original report of mold allergy in this country was that of Cadham[4] in 1924. He cited three cases of asthma from exposure to wheat rust (*Puccinia graminis Pers*) and demonstrated that symptoms arose after exposure to the rust and that skin tests to the rust extract were positive. Hopkins, Benham, and Kesten (1930)[5] reported a case of asthma from *Alternaria*. Extracts of several fungi recovered from the patient's cellar were made, and it was found that the patient gave a strong skin reaction to a species of *Alternaria*. Upon inhalation of a powder prepared by drying and grinding the washed pelt of an old broth culture of this fungus, a typical paroxysm of asthma resulted. A passage in the report deserves to be quoted: "The patient showed the most marked sensitiveness to *Alternaria* and this fungus also incited asthma. He showed, however, definite skin sensitiveness to *Cephalothecium, Paecilomyces,* and *Penicillia,* preparations of which failed to induce asthma. Conversely, one of the *Circinella* provoked asthma but failed to give a skin reaction. This lack of complete correlation is in accordance with numerous observations that skin reactions in asthmatic persons give only a lead toward the identification of the causative agent." This discrepancy between positive skin reactions and clinical sensitivity is still as important as when originally described by Hopkins, Benham, and Kesten.

Bernton[6] in 1930 reported asthma due to *Aspergillus fumigatus* and large skin tests by the scratch and intradermal methods were recorded. An attack of asthma occurred following a subcutaneous injection of an extract prepared from this fungus.

Flood[7] in 1931 precipitated an acute asthmatic paroxysm by spraying the throat with a broth filtrate prepared from *Mucor*.

Jimenez-Diaz, Cuenca, and Ping[8] (1932) in Madrid produced asthma in a patient by spraying her pillow with a culture of *Penicillium*, extracts of which had produced positive skin tests.

G. T. Brown[9] (1932) observed that the asthma in his patient was aggravated by the inhalation of spores while he was being skin tested with crude molds and dried pelt preparations.

There have been other reports of inhalant allergy from molds, the diagnosis being based on positive skin tests, but only in isolated instances has the clinical impression derived from the history and skin tests been augmented by a provocative clinical test.

The importance of the genus *Cladosporium* has been suspected on account of the high incidence of its spores in the air. In addition to Cobe's report previously cited, Bernton and Thom[10] in 1937 reported that among several hundred patients tested, four reacted to *Cladosporium* by scratch test with the ground dried pelt. Three cases had asthma and one rhinitis. Three of the four patients showed highly satisfactory results from treatment with the *Cladosporium* extract. Whether this extract contained more than one species of the genus *Cladosporium* or whether symptoms were produced upon artificial exposure to the mold culture is not stated.

We have observed many cases whose symptoms have occurred in association with dusts in sharply limited areas and whose histories otherwise suggest mold sensitivity, and we are now studying a group of such cases by testing them with autogenous mold extracts and attempting to induce attacks of asthma by exposure to the growth of these specific molds. It remains to be determined whether the high specificity as illustrated here in the genus *Cladosporium* is the rule or the exception in the inhalant allergy from fungi.

SUMMARY

1. The specificity of mold extracts, at least of species of *Cladosporium*, is demonstrated in a striking manner by intradermal tests made on four patients clinically sensitive to *Cladosporium fulvum Cke.*

2. In each of the four cases, asthma resulted from exposure to the spores of this mold, growing in a commercial tomato greenhouse.

3. Each patient reacted strongly to *Cladosporium fulvum Cke.* but showed little or no reaction to the other species of *Cladosporium* tested. Passive transfer of the skin test to normal persons was successful in each of the four cases.

4. The bearing of this finding on the study of fungous allergy is described.

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263 BEACON STREET, BOSTON

THE INFLUENCE OF EPINEPHRINE UPON ANTIGEN AND ANTIBODY ACTION*†

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IN ORDER to avoid possible untoward reaction from the administration of certain antigenic agents or immune antibody mixtures, the use of epinephrine either prior to or coincident with the injection has been advocated and is frequently employed with apparent success. Thus, for example, in the method of so-called desensitization prior to the administration of immune horse serum, it has been suggested[1] that epinephrine be given in small doses along with each fraction of serum. There is little question that this procedure is of value in the prevention of serious serum reaction. Likewise, in the administration of such substances as diphtheria toxoid or scarlet fever streptococcus toxin, it has been our practice as well as that of other physicians to administer 0.1 or 0.2 c.c. of epinephrine with every dose in order to prevent or minimize the possibility of unusual local or general reaction. In immunizing medical students or older children against diphtheria or scarlet fever, we have found that this procedure has often prevented subsequent constitutional reactions and minimized the severity of the local reactions which are more frequent in these individuals than in younger children.

The question has arisen at times as to whether the inclusion of the epinephrine along with either the antigen or the antibody would act in any way to inhibit its immunologic action. This seemed very unlikely, at least from a theoretical standpoint, and also when one considers the pharmacologic action of this drug. Review of the available literature failed to supply a satisfactory answer to this question. It seemed worth while, therefore, to attempt to settle it more definitely by means of the following experiments:‡

PROCEDURE

Twelve guinea pigs, divided into four series of three each were employed in the experiment. Each series was treated as follows:

Series 1: Each animal was injected subcutaneously with 1 c.c. of a solution containing diluted diphtheria toxin in the equivalent of the L+ dose.

*Read before the Philadelphia Allergy Society, October 27, 1937.
†From the Allergy Clinic, Temple University Hospital and School of Medicine, and the Research Institute of Cutaneous Medicine.
‡For technical assistance in the performance of these experiments, the author is grateful to Miss Anna M. Rule of the Research Institute of Cutaneous Medicine.

Series 2: These animals were given the same amount of toxin except that 0.1 c.c. of 1:1000 epinephrine solution was added to each dose.

Series 3: Each animal was given 1 c.c. of the diluted toxin containing the L+ dose plus 1 c.c. of a sufficient amount of diluted diphtheria antitoxin to equal 1 unit.

Series 4: These animals were given the same mixture as in Series 3, except that 0.1 c.c. of 1:1000 epinephrine was added to each dose.

The time of injection and the time of the death of the animals were noted as carefully as possible. From previous experience with the use of these agents in the standardization of diphtheria antitoxin,* it had already been determined that if epinephrine had no effect upon the antigenic action of the diphtheria toxin, death should normally occur within forty-eight hours for the animals in Series 1 and 2, and within four days for those in Series 3 and 4. In the latter instance, the action of the antitoxin was indicated by a delay in the death of the animals.

RESULTS

These are summarized in the following table:

TABLE I

SERIES	MATERIAL INJECTED	DEATH OF ANIMALS IN HOURS		
		NO. 1	NO. 2	NO. 3
1 (Control)	Diphtheria Toxin (L+ dose)	30	35	40
2	Same as 1, plus 0.1 c.c. epinephrine	30	39	48
3 (Control)	Diphtheria Toxin (L+ dose) plus Diphtheria Antitoxin (1 unit)	48	54	60
4	Same as 3, plus 0.1 c.c. epinephrine	48	60	72

All the animals in Series 1 and 2 appeared quite sick at the end of twenty-four hours. This greatly increased until death occurred. The three animals in Series 1, the control group, died in thirty, thirty-five, and forty hours, respectively, whereas those in Series 2 that received epinephrine died in thirty, thirty-nine, and forty-eight hours, respectively. In both groups, therefore, death had occurred within the forty-eight-hour period, as was to be expected after the administration of the L+ dose of diphtheria toxin. The animals in Series 3 and 4 all looked well up to thirty-six hours, then became sick in increasing measure until death finally ensued. The animals in the control group, Series 3, died in forty-eight, fifty-four, and sixty hours, respectively, whereas those in Series 4, which received toxin-antitoxin and epinephrine died in forty-eight, sixty, and seventy-two hours, respectively. The time of death in Series 3 and 4 likewise was in accordance with what would normally be expected.

*The standardized toxin and antitoxin employed in these experiments were provided through the courtesy of the Mulford Biological Laboratories of Sharp & Dohme, Glenolden, Pa.

COMMENT

It is apparent from a review of these results that the only effect produced by the addition of epinephrine to the toxin or antitoxin mixture, was to cause slight delay in the death of the animals as compared to the control group. It did not apparently alter or interfere with the immunologic action of either the antigenic or antibody components of the mixture. The final effects of these agents were evident in spite of the slight delay occasioned by the epinephrine.

This effect substantiates previous clinical belief and is in accordance with what one would expect from a knowledge of the pharmacologic action of epinephrine. It would seem likely that the delay in the absorption of these immune agents* produced by the drug is the result of its marked local vasoconstricting action. Since it apparently does not interfere with the immunologic action of these substances, its use is indicated and valuable in the prevention of constitutional or severe local reactions. The epinephrine can be given in doses of 0.1 to 0.2 c.c. along with the immune agent or administered separately above the site of injection of the latter. We have employed both methods but prefer to add the drug directly to the immune substance before injecting it. As mentioned previously we have found this procedure of value in our clinic in immunizing medical students against scarlet fever or diphtheria, a small amount of epinephrine being included with each dose.

It is possible, of course, that the intradermal method of immunization against scarlet fever or diphtheria as suggested by Kern and his coworkers[3, 4] may obviate the necessity of employing epinephrine in this way, but until their experiments are confirmed and the method more widely employed, the addition of this drug to each dose of either diphtheria toxoid or scarlet fever streptococcus toxin is advisable either as a routine procedure or whenever there is fear of a possible reaction from the injection of any type of immune agent—thus we have also employed it to advantage in giving pollen extracts to individuals who are prone to develop constitutional reactions especially during the pollinating season. Likewise, as mentioned at the outset, it is very useful when given in combination with the fractional doses of serum recommended in the method of so-called "desensitization" in patients sensitive to horse serum.[1] It must be emphasized, of course, that its employment should not convey a complete sense of security against possible reaction, but when used judiciously, it can be very valuable in entirely preventing or at least in minimizing this possibility.

*This is further substantiated in a recent article by Feinberg and Bernstein[2] in which it is demonstrated that epinephrine acts upon pollen extracts by decreasing their absorption rather than interfering with their reaction.

CONCLUSION

Experimental study of the effect of the addition of epinephrine to antigen or antibody mixtures indicates that it produces delay in the absorption of these agents but does not interfere with their specific immunologic action. This effect makes its use valuable in preventing or minimizing unusual local or marked constitutional reactions produced by the administration of such agents as diphtheria toxoid, scarlet fever streptococcus toxin, or pollen extracts employed in active immunization, or to prevent reactions when carrying out so-called "desensitization" in serum-sensitive individuals.

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A METHOD FOR DETERMINING POLLEN CONCENTRATION OF THE AIR*

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IN A RECENT paper the author (Cocke[1]) pointed out and corrected an error in Scheppegrell's formula for computing pollen concentration of the atmosphere. Scheppegrell's formula was based on Stokes's law for falling bodies, the correctness of which is undisputed. The corrected figures for Scheppegrell's formula should then give an accurate indication of the true concentration per cubic yard of air provided proper allowance is made for differences in size of various pollen grains. It must be remembered also that Stokes's law holds for smooth spherical bodies only; with rough or spiculated pollen the velocity of fall is considerably less.

It is the purpose of this paper to describe a method for measuring the number of pollen grains per cubic yard and compare the results obtained

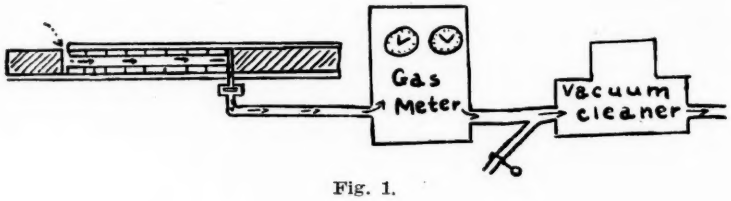

Fig. 1.

by actual measurement with calculated results. A table for determining the number of pollen per cubic yard from the number caught on 1 square centimeter in twenty-four hours is also included.

METHODS

The apparatus for collecting the pollen from a measured amount of air is diagrammed in Fig. 1. It consists of a small chamber lined with microscopic slides which have been smeared with glycerine jelly, a gas meter for measuring the amount of air passing through the chamber, and a vacuum cleaner for sucking the air through the chamber. The chamber was made by cutting a rectangular hole approximately 3×8 inches in a piece of glass $8 \times 12 \times \frac{3}{16}$ inches. To the bottom of this, a 10×12 inch window glass was fastened with Canada balsam. A $\frac{3}{8}$ inch hole bored through the bottom at one end of the chamber, midway between the sides of the chamber, provided an outlet. The top of the chamber con-

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sisted of a piece of window glass placed over the chamber and sealed with vaseline. A space about 2 mm. wide was left at the end of the chamber away from the outlet. The outlet tube was connected to the meter, which was in turn connected to the vacuum cleaner. The connection between the meter and vacuum cleaner was provided with a "Y" tube with a valve for regulating the speed with which air was drawn through the chamber. When the apparatus was ready to use, eight 3 × 1 inch slides smeared with glycerine jelly were placed in the bottom of the chamber, and an equal number of similarly prepared slides were fastened to the top of the chamber with vaseline in such a manner as to fit directly into the chamber. The space between the upper and lower slides could easily be adjusted by putting thin pieces of glass 3 × 8 in. in the bottom. It was found that a space of about 1 to 2 mm. gave most satisfactory results. The speed of the air passing through the chamber was regulated so that the air in the chamber was changed approximately once each second. This speed allowed the pollen ample time to settle out in passing the 8 inch distance from entrance to outlet.

The apparatus was used for a total of approximately twenty-four hours at varying times of the day during three periods of the pollen season, May, June, and August. During this time approximately 50 cubic feet of air was measured. The slides were changed at the end of each running period and the grains identified and counted. A mechanical stage was used and care taken to count all of the grains. Slides smeared with glycerine jelly were exposed simultaneously with each testing period, and the number of grains counted and the pollen concentration calculated from Cocke's revision of Scheppegrell's formula.

The results obtained by the two methods of determining the pollen concentration are given in the following table:

TABLE I

KIND OF POLLEN	SIZE IN MICRONS	CONCENTRATION PER CUBIC YARD			
		COMPUTED BY GRAVITY METHOD	ACTUAL NO. CAUGHT PER CUBIC YD.	INCREASE OR DECREASE OF NO. CAUGHT OVER COMPUTED NUMBER	PERCENTAGE INCREASE OR DECREASE
Broussonetia	14	6240	5904	−336	− 5.4
Ambrosia	18	1740	2300	+560	+32.2
Salix	18	73	64	− 9	−12.3
Platanus	22	80	86	+ 6	+ 7.5
Morus	22	256	270	+ 14	+ 5.5
Maclura	24	75	75	0	0
Quercus	28	1961	2130	+169	+ 8.6
Rumex	30	80	81	+ 1	+ 1.3
Plantago	30	50	57	+ 7	+14.0
Betula	32	96	83	− 13	−13.5
Juglans	40	49	41	− 8	−16.0
Liquidamber	40	72	75	+ 3	+ 4.1
Grass	40	21	17	− 4	−19.0
Carya	45	65	59	− 6	− 9.0
Pinus	50	320	490	+170	+56.2

TABLE II

FOR DETERMINING THE NUMBER OF POLLEN GRAINS OF A GIVEN SIZE PER CUBIC YARD OF AIR FROM THE NUMBER OF GRAINS CAUGHT ON ONE SQUARE CENTIMETER IN TWENTY-FOUR HOURS

	12μ	14μ	16μ	18μ	20μ	22μ	24μ	26μ	28μ	30μ	32μ	34μ	36μ	38μ	40μ	42μ	44μ	46μ	48μ	50μ
1	21	17	11	10	7	6	5	4	4	3	3	3	2	2	2	2	2	1	1	1
2	42	33	22	19	15	12	10	9	7	6	6	5	4	4	4	3	3	3	3	2
3	62	50	34	29	22	18	15	13	11	10	9	8	7	6	5	5	5	4	4	4
4	83	67	45	38	29	23	20	17	15	13	12	11	9	8	7	6	6	6	5	5
5	104	83	57	48	37	29	25	21	18	16	14	13	11	10	9	8	8	7	7	6
6	125	100	67	58	43	35	30	26	22	19	17	16	13	11	11	10	9	8	8	7
7	145	116	78	68	51	41	35	30	26	22	20	19	15	13	13	11	11	10	9	8
8	166	132	90	78	58	47	40	34	30	26	23	21	18	15	14	13	12	11	10	10
9	187	149	101	87	66	53	45	39	33	29	26	24	20	17	16	14	14	13	12	11
10	208	166	112	97	73	59	50	43	37	32	30	27	22	19	18	16	15	14	13	12
11	229	183	123	107	80	65	55	47	40	35	33	30	24	21	20	18	17	15	14	13
12	250	199	135	116	88	71	60	52	54	38	35	32	26	23	22	19	18	17	16	14
13	270	216	144	126	95	77	65	56	48	42	38	35	29	25	23	21	20	18	17	16
14	281	232	157	136	102	83	70	60	52	45	41	38	31	27	25	22	21	20	18	17
15	312	249	168	146	109	89	75	64	55	48	44	41	33	29	27	24	23	21	20	18
16	333	266	179	155	117	95	80	69	59	51	46	43	35	30	29	26	24	22	21	19
17	354	282	190	165	124	101	85	73	63	54	49	46	37	32	31	27	26	24	22	20
18	374	299	201	185	131	106	90	77	67	58	53	49	40	34	32	29	27	25	23	22
19	395	315	212	184	139	112	95	82	70	61	55	51	42	36	34	30	29	27	25	23
20	416	333	223	194	146	118	100	86	74	64	58	54	44	38	36	32	30	28	26	24
21	437	349	235	204	153	124	105	90	78	67	61	57	46	40	38	34	32	29	27	25
22	458	365	246	214	160	130	110	94	81	70	64	60	49	42	40	35	33	31	29	26
23	479	382	258	224	167	136	115	99	85	74	67	62	51	44	41	37	35	32	30	28
24	500	399	269	234	175	142	120	103	89	77	70	65	53	46	43	38	36	34	31	29
25	520	415	280	242	143	147	125	108	92	80	73	68	55	48	45	40	38	35	33	30
26	541	432	291	251	190	153	130	112	96	83	75	70	57	49	47	42	39	36	34	32
27	562	448	302	261	197	159	135	116	100	86	78	73	59	51	49	43	41	38	35	32
28	582	465	314	271	204	165	140	120	104	90	81	76	62	53	50	45	42	39	36	34
29	603	481	325	280	212	171	145	125	107	93	84	76	64	55	52	46	44	41	38	35
30	624	498	336	290	219	177	150	129	111	96	87	81	66	57	54	48	45	42	39	36

Directions for using Table II are given in the last paragraph of the discussion.

TABLE II—CONT'D

31	645	515	347	300	226	183	155	133	115	99	90	84	68	59	56	50	47	43	40	37
32	666	531	358	309	234	189	160	138	118	102	93	86	70	61	58	51	48	45	42	38
33	686	548	370	319	241	195	165	142	122	106	96	89	73	63	59	53	50	46	43	40
34	707	564	381	329	248	201	170	146	126	109	99	92	75	65	61	54	51	48	44	41
35	728	581	392	339	256	207	175	151	130	112	102	95	77	67	63	56	53	49	46	42
36	749	598	403	348	263	212	180	155	133	115	104	97	79	68	65	58	54	50	47	43
37	770	614	414	358	270	218	185	159	137	118	107	100	81	70	67	59	56	52	48	44
38	790	631	426	368	277	224	190	163	141	122	110	103	84	72	68	61	57	53	49	46
39	811	647	437	371	285	230	195	168	144	125	113	105	86	74	70	62	59	55	51	47
40	832	664	448	387	292	236	200	172	148	128	116	108	88	76	72	64	61	56	52	48
41	853	680	459	397	299	242	205	176	152	131	119	111	90	78	74	66	62	57	53	49
42	874	697	470	406	307	248	210	181	155	134	122	113	92	80	75	67	63	59	55	50
43	894	714	482	416	314	254	215	185	159	138	125	116	95	82	77	69	65	60	56	52
44	915	730	493	426	321	260	220	189	163	141	128	119	97	84	79	70	66	62	57	53
45	936	747	504	436	329	266	225	194	167	144	131	122	99	86	81	72	68	63	59	54
46	957	764	515	445	336	271	230	198	170	147	133	124	101	87	83	74	69	64	60	55
47	978	780	526	455	343	277	235	202	174	150	136	127	103	89	84	75	71	66	61	56
48	998	797	538	462	350	283	240	206	178	154	139	130	106	91	86	77	72	67	62	58
49	1019	813	549	474	358	289	245	211	181	157	142	132	108	93	88	78	74	69	64	59
50	1040	830	560	484	365	295	250	215	185	160	145	135	110	95	90	80	75	70	65	60
51	1061	847	571	494	372	301	255	219	189	163	148	138	112	97	92	82	77	71	66	61
52	1082	863	582	503	380	307	260	224	192	166	151	140	114	99	93	83	78	73	68	62
53	1102	880	594	513	387	313	265	228	196	170	154	143	117	101	95	85	80	74	69	64
54	1123	896	605	523	394	319	270	232	200	173	157	146	119	103	97	86	81	76	70	65
55	1144	913	616	533	402	325	275	237	204	176	160	149	121	105	99	88	83	77	72	66
56	1165	930	627	542	409	330	280	241	207	179	162	151	123	106	101	90	84	78	73	67
57	1186	946	638	552	416	336	285	245	211	182	165	154	125	108	102	91	86	80	74	68
58	1206	963	650	562	423	342	290	249	215	186	168	157	128	110	104	93	87	81	75	70
59	1227	979	661	571	431	348	295	254	218	189	171	159	130	112	106	94	89	83	77	71
60	1248	996	672	581	438	354	300	258	222	192	174	162	132	114	108	96	90	84	78	72
61	1269	1013	683	591	445	360	305	262	226	195	177	165	134	116	110	98	92	85	80	73
62	1290	1029	694	600	453	366	310	266	229	198	180	167	136	118	111	99	93	87	81	74
63	1310	1046	706	610	460	372	315	271	233	202	183	170	139	120	113	101	95	88	82	76
64	1331	1062	717	620	467	378	320	275	237	205	186	173	141	122	115	102	96	90	83	77
65	1352	1079	728	630	475	384	325	280	241	208	189	176	143	124	117	104	98	91	85	78

TABLE II—CONT'D

	12μ	14μ	16μ	18μ	20μ	22μ	24μ	26μ	28μ	30μ	32μ	34μ	36μ	38μ	40μ	42μ	44μ	46μ	48μ	50μ
66	1373	1096	739	639	482	389	330	284	244	211	191	178	145	125	119	106	99	92	86	79
67	1394	1112	750	649	489	395	335	285	248	214	194	181	147	127	120	107	101	94	87	80
68	1414	1129	762	659	496	401	340	293	252	218	197	184	150	129	122	109	102	95	88	82
69	1435	1145	773	668	504	407	345	296	255	221	200	186	152	131	124	110	104	97	89	83
70	1456	1162	784	678	511	413	350	301	259	224	203	189	154	133	126	112	105	98	91	84
71	1477	1179	795	688	518	419	355	305	263	227	206	192	156	135	128	114	107	99	92	85
72	1498	1195	806	697	526	425	360	310	266	230	209	194	158	137	129	115	108	101	94	86
73	1518	1212	818	707	533	431	365	314	270	234	212	197	161	139	131	117	110	102	95	88
74	1539	1228	829	717	540	437	370	318	274	237	215	200	163	141	133	118	111	104	96	89
75	1560	1245	840	727	548	443	375	323	278	240	218	203	165	143	135	120	113	105	98	90
76	1581	1261	851	736	555	448	380	327	281	243	220	205	167	144	137	122	114	106	99	91
77	1602	1278	862	746	562	454	385	331	285	247	223	208	169	146	138	123	116	108	100	93
78	1622	1295	874	756	569	460	390	335	289	250	226	211	172	148	140	125	117	109	101	94
79	1643	1311	885	765	577	466	395	340	292	253	229	213	174	151	142	126	119	111	103	95
80	1664	1328	896	776	584	472	400	344	296	256	232	216	176	152	144	128	120	112	104	96
81	1685	1345	907	786	591	478	405	348	300	259	235	219	178	154	146	130	122	113	105	97
82	1706	1361	918	795	599	484	410	353	303	262	238	221	180	156	147	131	123	115	107	98
83	1726	1378	930	805	606	490	415	357	307	266	241	224	183	158	149	133	125	116	108	100
84	1747	1394	941	815	613	496	420	361	311	269	244	227	185	160	151	134	126	118	109	101
85	1768	1411	952	825	621	502	425	366	315	272	247	230	187	162	153	136	128	119	111	102
86	1789	1428	963	834	628	507	430	370	318	275	249	232	189	163	155	137	129	120	112	103
87	1810	1444	974	844	635	513	435	374	322	278	252	235	191	165	156	139	131	122	113	104
88	1830	1461	986	854	642	519	440	378	326	281	255	238	194	167	158	141	132	123	114	106
89	1851	1477	997	863	650	525	445	383	329	285	258	240	196	169	160	142	134	125	116	107
90	1872	1494	1008	873	657	531	450	387	333	288	261	243	198	171	162	144	135	126	117	108
91	1893	1511	1019	883	664	537	455	391	337	291	264	246	200	173	164	146	137	127	118	109
92	1914	1527	1030	892	672	543	460	396	340	294	267	248	202	175	165	147	138	129	120	110
93	1934	1544	1042	902	679	549	465	400	344	298	270	251	205	177	167	149	140	130	121	112
94	1955	1560	1053	912	686	555	470	404	348	301	273	254	207	179	169	150	141	132	122	113
95	1976	1577	1064	922	694	561	475	409	352	304	276	257	209	181	171	152	143	133	124	114
96	1997	1594	1075	931	701	566	480	413	355	307	278	259	211	182	173	153	144	134	125	115
97	2018	1610	1086	941	708	572	485	417	359	310	281	262	213	184	174	155	146	136	126	116
98	2038	1627	1098	951	715	578	490	421	363	315	284	265	215	186	176	157	147	137	127	118
99	2059	1643	1109	960	723	584	495	426	366	317	287	267	217	188	178	158	149	139	128	119
100	2080	1660	1120	970	730	590	500	430	370	320	290	270	220	190	180	160	150	140	130	120

DISCUSSION

The pollen concentration determined by actual experiment is strikingly close to those derived by the gravity method. As is expected in the spiculated pollen of ragweed, the concentration determined by actual count is considerably higher than that calculated from the gravity method. The increase in oak, sweet gum, and sycamore is also expected.

The wings or air sacs on the pine pollen explain its low velocity of fall, and, therefore, the great increase of the actual count over the calculated results. Other increases and decreases cannot be explained definitely at present. A careful determination of the specific gravity of the various pollens will no doubt shed much light on the question.

For the convenience of workers on atmospheric pollen Table II is included. This table furnishes an easy method for determining the pollen concentration from the number of grains caught on 1 sq. cm. in twenty-four hours. The number of grains caught is given in the vertical column on the left while the size of the grains is given across the page. The application of the table is simple. For example, if one catches 28 giant ragweed pollens (size 20 μ) on 1 sq. cm. in twenty-four hours, he reads 28 in the vertical column to the left and then over to the column headed 20 μ and the answer is 204. Since ragweed is spiculated, he should then add one-third of the number, which gives 272 as the actual concentration.

SUMMARY

1. The gravity method for determining pollen concentration of the atmosphere gives a true picture of the actual pollen concentration as shown by actual experiment.

2. For spiculated pollen such as ragweed, the actual concentration is about one-third greater than the calculated concentration.

3. For pollen grains with air sacs or wings, 50 per cent should be added to the calculated atmospheric concentration.

4. A table for calculating pollen circulation from the number of grains caught on 1 sq. cm. in twenty-four hours is appended.

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1. Cocke, Elton C.: Calculating Pollen Concentration of the Air. J. ALLERGY 8: 601, 1937.
2. Scheppegrell, Wm.: Hayfever and Asthma, New York City, 1922, Lea & Febiger.

STUDIES WITH ANTIGENS*

I. The Skin Reaction Curve Obtained With Serial Dilutions of Extracts

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DIRECT skin tests for the determination of the antigenic potency of extracts are in constant use. They are called upon whenever it is desired to ascertain the relative potency of two extracts of the same substance. The advocates of the chemical standardization of extracts use this method of testing for the validity or superiority of their particular technique. Also, the stability of extracts has been gauged from the results of comparative skin tests. Various extracting solutions have been advocated as having superior merit as the result of the findings by these tests. Bowman[1] reports that by the utilization of a very careful technique, particularly with reference to placing and alternating the test sites, differences in reaction size may be detected in only the majority of cases when one pollen extract is 25 per cent stronger in potency than the other; but that these differences are not detectable when this technique is not followed.

The present accepted significance of comparative skin tests is based upon two propositions: (1) that in a given subject, a given size of skin reaction between the minimal and maximal, can be produced by extract potencies within one and only one range. This means that equal-sized skin reactions denote potencies within a small range of difference; (2) that extract potency, test location on the skin, and identity of subject are the only factors affecting the size of skin reactions.

Comparative skin tests as hitherto performed and interpreted, failed to demonstrate, in most instances, constant or significant differences in potency of the various fractions obtained by us during ultrafiltration and other experiments. Stull, Cooke, and Tennant[2] state, "In many instances, it was impossible to determine differences in the strength of extracts by direct skin tests since frequently extracts 10 and even 100 times stronger by dilution, gave identical skin reactions when readings were made within 30 minutes."

Various factors influence the size of the skin reaction. Alexander and McConnell[3] showed a constant variation in the size of the reaction when the test was performed on different parts of the body. Larsen, Paddock, and Alexander[4] found that reactions produced by tests of like amount

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and concentration on the forearm decreased from the elbow to the wrist. Bowman showed that reactions on the upper part of the arm tended to be larger than those on the lower, and that reactions on the medial side of the arm tended to be larger than those on the lateral. With the scratch technique, the size and depth of the scratch are important factors influencing the size of the reaction.

METHOD

Scratch tests were performed only on the forearms. Scratches about 3/8 inch long and about 1 inch apart were made, so that no more than four scratches were made on the forearm of adults, and no more than three on each forearm in children. Reactions were read at the end of twenty minutes.

The intracutaneous tests were carefully done. They were made vertically on the lateral aspect of the arm. As nearly as possible, equal amounts of extract were injected into the test sites. Injections were made as superficially as possible; approximately 0.01 c.c. was injected into each site. Tests were placed at least two inches apart. Reactions were read at the end of twelve minutes.

Since only a few concentrations could be tested at one visit, tests were made on subsequent days and were usually started at least with the last concentration of that given on the previous visit. At times, tests were started with one or two concentrations above the last one tested. This was done intentionally in order to minimize as far as possible the reactivity difference between the skin at the elbow and wrist and between the upper and lower part of the arm, so that many dilutions were tested several times during the course of the biologic assay on a patient. This will help to explain the different sized reactions with the same dilution noted at times on the same patient. In many cases the whole series of tests was repeated. The order with which the extracts were tested was always from the more concentrated to the less concentrated.

The reactions* were recorded as marked plus (M+), marked (M), marked moderate (Mm), moderate (Mo), moderate slight (Ms), slight (S), plus minus slight (± S), and plus minus or negative (± or 0). Any other practical recording system could have been adopted. However, one with many classifications of reactions was purposely chosen, in order that slight variations in the size of the reactions would be noted. A system of recording that would have had fewer classifications would have placed many more reactions in the same group than did this system. On the other hand, a system with more classifications would be impractical.

*The reactions were classified as follows: *M+*, wheal with pseudopods, 10 mm. or more diameter, large erythema; *M*, wheal with pseudopods, 8.5 to 9.5 mm. diameter, large erythema; *Mm*, wheal with pseudopods, 7.5 to 8.0 mm. diameter, large erythema; *Mo*, wheal with no pseudopods, or very slight pseudopod formation, 5 to 7 mm. diameter, erythema; *Ms*, wheal with no pseudopods, 3.5 to 4.5 mm. diameter, erythema; *S*, wheal with no pseudopods, 2 to 3 mm. diameter, erythema; and ±*S*, no wheal formation, erythema only.

House Dust.—House dust extract was used only with the scratch technique, and diluted serially. To each one part of dust extract was added the following parts of Coca's solution and labeled as follows:

TABLE I

DILUTION	DUST EXTRACT PARTS	COCA SOLUTION PARTS	PERCENTAGE OF ORIGINAL CONCENTRATION AS USED IN GRAPHS
ORIGINAL DUST EXTRACT	1	0	100
$\frac{D}{1.25}$	1	0.25	80
$\frac{D}{1.50}$	1	0.50	$66\frac{2}{3}$
$\frac{D}{2}$	1	1.00	50
$\frac{D}{2.5}$	1	1.50	40
$\frac{D}{3}$	1	2.00	$33\frac{1}{3}$
$\frac{D}{4}$	1	3.00	25
$\frac{D}{5}$	1	4.00	20
$\frac{D}{6}$	1	5.00	$16\frac{2}{3}$
$\frac{D}{7}$	1	6.00	$14\frac{1}{3}$
$\frac{D}{8}$	1	7.00	$12\frac{1}{2}$
$\frac{D}{9}$	1	8.00	11
$\frac{D}{10}$	1	9.00	10
$\frac{D}{11}$	1	10.00	9
$\frac{D}{13}$	1	12.00	$7\frac{1}{2}$
$\frac{D}{15}$	1	14.00	$6\frac{2}{3}$
$\frac{D}{20}$	1	19.00	5
$\frac{D}{25}$	1	24.00	4
$\frac{D}{30}$	1	29.00	$3\frac{1}{3}$
$\frac{D}{40}$	1	39.00	$2\frac{1}{2}$
$\frac{D}{50}$	1	49.00	2
$\frac{D}{60}$	1	59.00	$1\frac{2}{3}$

The results of the tests are represented by graphs. The curves are not composite, but actual curves, for the reactions obtained. The graphs were made as follows: the concentrations of the test solutions used were

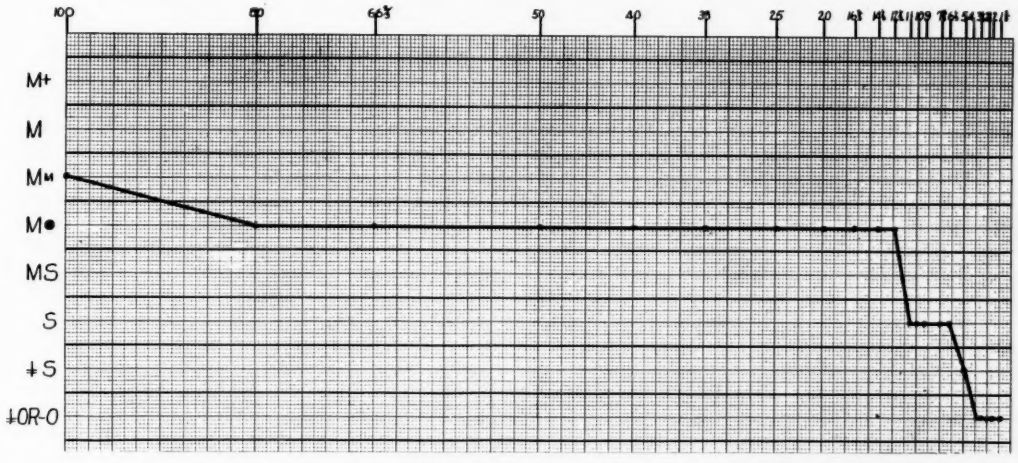

Fig. 1.—A diminution in concentration of 20 per cent* between 100 and 80 shows a difference in the size of the reactions, but an 84 per cent diminution in concentration between 80 and 12½† fails to show a difference in reaction size. There is a marked difference in the size of the reactions produced at 12½ and 11, a concentration diminution of only 12 per cent; another plateau of equal sized reactions occurs between 11 and 1⅔, a diminution in concentration of 39 per cent.

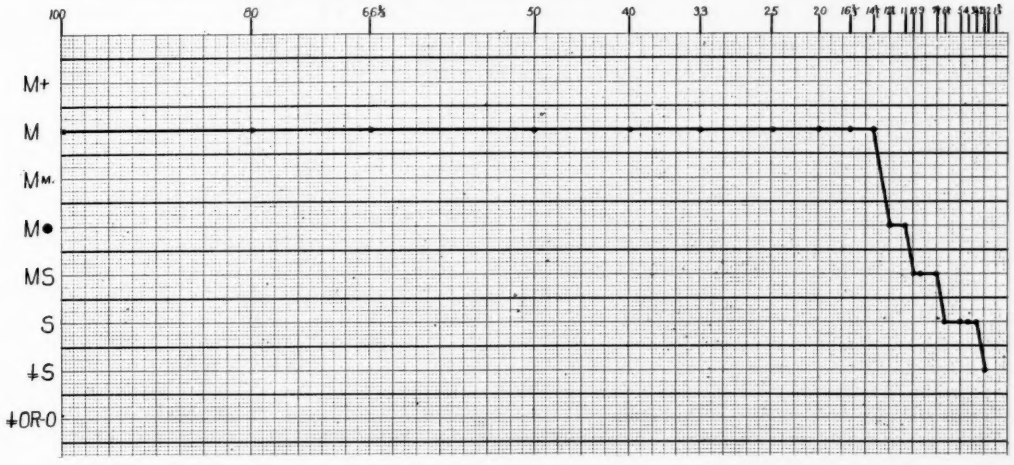

Fig. 2.—One hundred is 85⅞ per cent more concentrated than 14⅓, yet the reactions are similar for this great diminution in concentration. A 13 per cent diminution in concentration between 14⅓ and 12½ produces a difference in reactions, as does a 9 per cent diminution between 11 and 10; a plateau occurs between 10 and 7½; another plateau between 6⅔ and 3⅓ (a 50 per cent diminution in concentration of the extract).

expressed in percentages of the original dust extract, taken as 100; the reactions were classified into eight sizes, including the maximal (M+) and the minimal (± or 0).

*If the graphs are read from the point of view of increasing instead of decreasing concentrations, the percentages are very much larger; the facts remain the same, although the larger percentage figures are more dramatic; for example, the 84 per cent diminution in concentration between 80 and 12½ becomes a 540 per cent increase in concentration from 12½ to 80.

†For convenience of charting, fractions of per cent are used rather than decimals.

The reaction curve with dust extract was completed in 24 cases. Examination of Figs. 1, 2, 3, and 4, representing the skin reaction curve obtained with serial dilutions of house dust extract, shows distinctly that the curve is discontinuous. Extracts six, five, and four times, etc.,

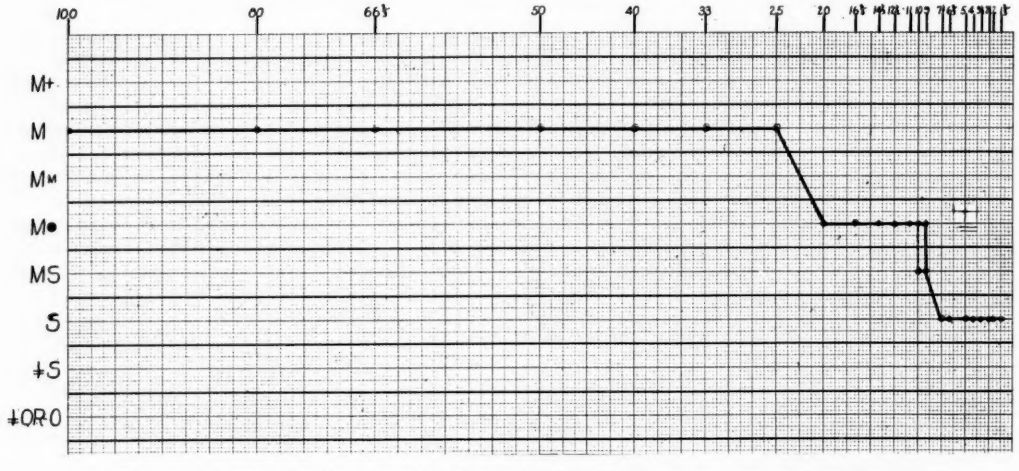

Fig. 3.—Similar reactions persist although 100 is 4 times as concentrated as 25; a diminution in concentration of 20 per cent between 25 and 20, results in a diminution in the size of the reaction; a plateau of reaction sizes occurs between 20 and 10 or 9, depending on whether the upper or lower curve is read (diminution in concentration of 50 per cent to 55 per cent. Note 10 and 9, where different reactions occur for identical concentrations. Note plateau of slight reactions between 7⅛ and 1⅔, a diminution in concentration of 76 per cent. These reactions persist to the end of the range of dilutions tested. This persistence of slight reactions with high dilutions is not an uncommon finding with scratch tests.

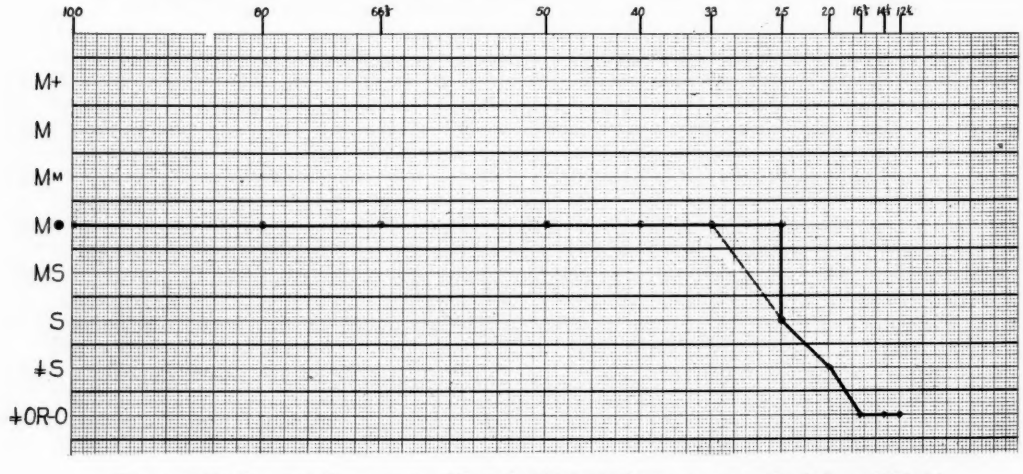

Fig. 4.—Similar reactions occur although 100 is 3 times as concentrated as 33⅓ (4 times as concentrated as 25). Note 25, where different reactions were obtained for the same concentrations. The larger reaction occurred when the test site was near the elbow, the smaller when it was near the wrist. Note also the comparatively rapid drop to negative reactions.

as concentrated produce identical sized skin reactions, whereas slight diminutions in concentrations, such as 9 per cent, 12 per cent, etc., result in decreases in the size of the reactions. The curve shows an overwhelming tendency toward similar sized reactions in the upper ranges

of concentration of the extract. Plateaus of equal sized reactions occur, however, in all ranges of dilution. Extracts of equal concentration occasionally produce unequal sized reactions.

Bermuda Grass.—A. Scratch Tests. A 3 per cent (weight to volume) extract of Bermuda grass pollen was used for the scratch tests. The

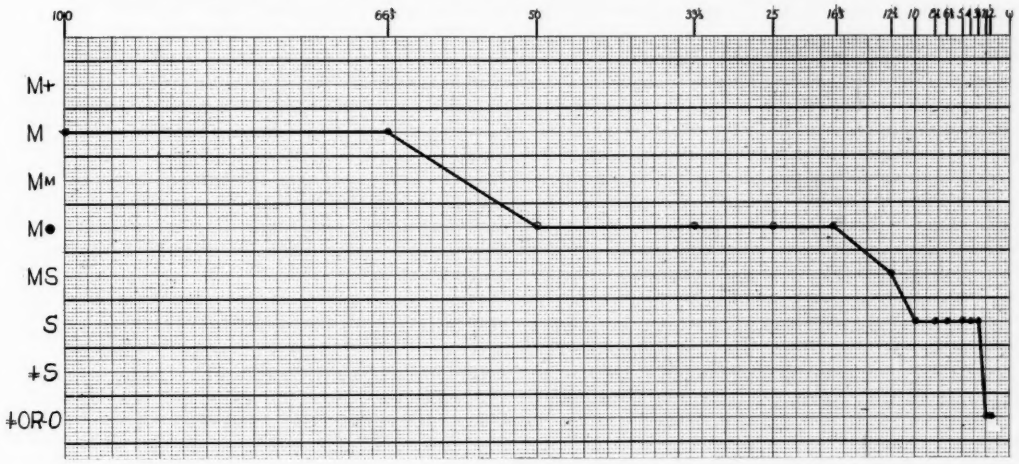

Fig. 5.—Equal reactions occur between 100 and 66⅔; a diminution in concentration of 33⅓ per cent; a diminution of 25 per cent produces different reactions (66⅔ and 50), but from 50 through 16⅔, a plateau of reactions occurs, and 50 is 67 per cent more concentrated than 16⅔; another difference in reaction size occurs between 16⅔ and 12½, and between 12½ and 10; followed by a plateau of similar sized reactions between 10 and 2½ (10 is 75 per cent more concentrated than 2½).

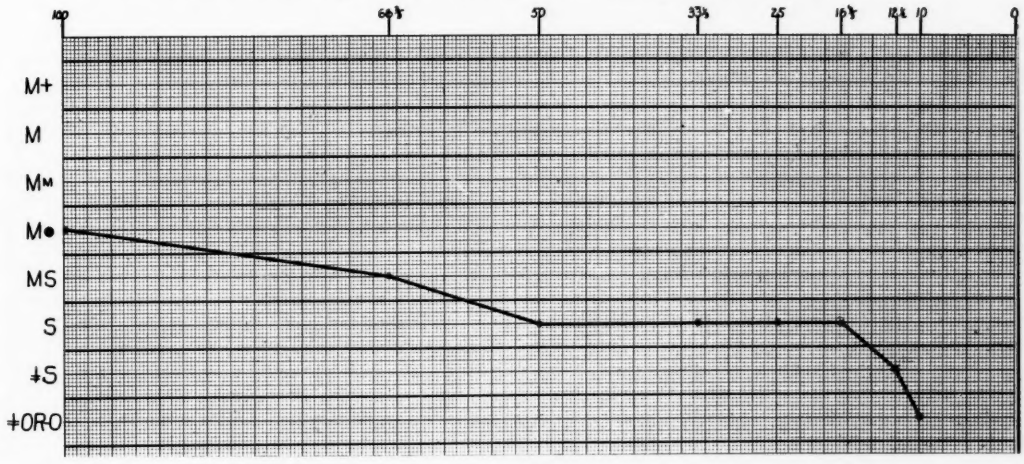

Fig. 6.—One hundred and 66⅔ produce different reactions, as do 66⅔ and 50, but a plateau of reactions occurs between 50 and 16⅔ (50 is 3 times as concentrated as 16⅔); then again a difference in reactions is obtained between 16⅔ and 12½, and between 12½ and 10.

following dilutions were used for the series of tests: Grass 3 per cent, Gr. 1.5, Gr. 2, Gr. 3, Gr. 4, Gr. 6, Gr. 8, Gr. 10, Gr. 12, Gr. 15, Gr. 20, Gr. 25, Gr. 30, Gr. 40, Gr. 50, and Gr. 60. The dilutions were made by the technique described above for the house dust

extract, and likewise rated in the graphs as percentage concentrations of the original extract, the original 3 per cent extract being taken as 100.

The reaction curves for the grass extract are represented by Figs. 5, 6, 7, and 8. No graphs essentially different from those presented

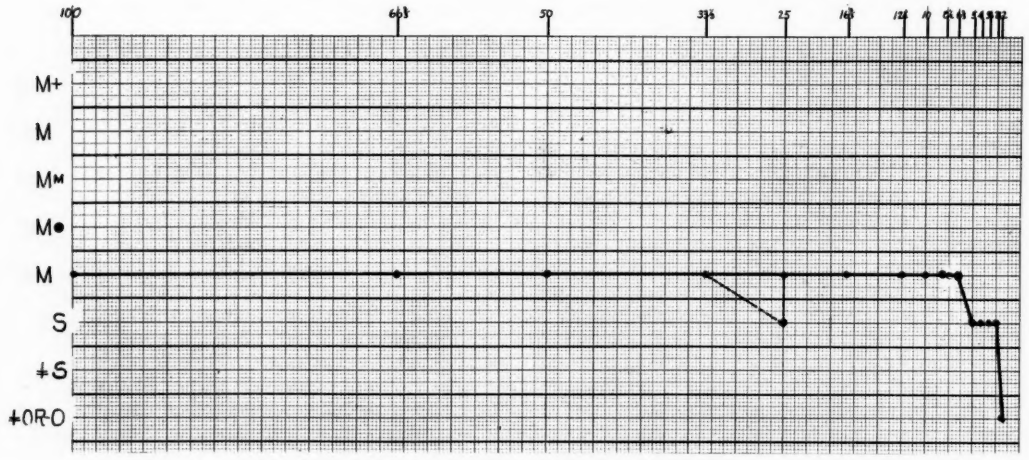

Fig. 7.—A plateau of reaction sizes occurs between 100 and 33⅓ if the lower curve is read (100 is 67 per cent more concentrated than 33⅓) or between 100 and 6⅔ if the upper curve is read (a difference in concentration of 93 per cent). Between 6⅔ and 5 there is a diminution in reaction size, with another plateau between 5 and 2½ (a diminution in concentration of 50 per cent), followed by a marked diminution in reactions between 2½ and 2 (a diminution in concentration of only 20 per cent).

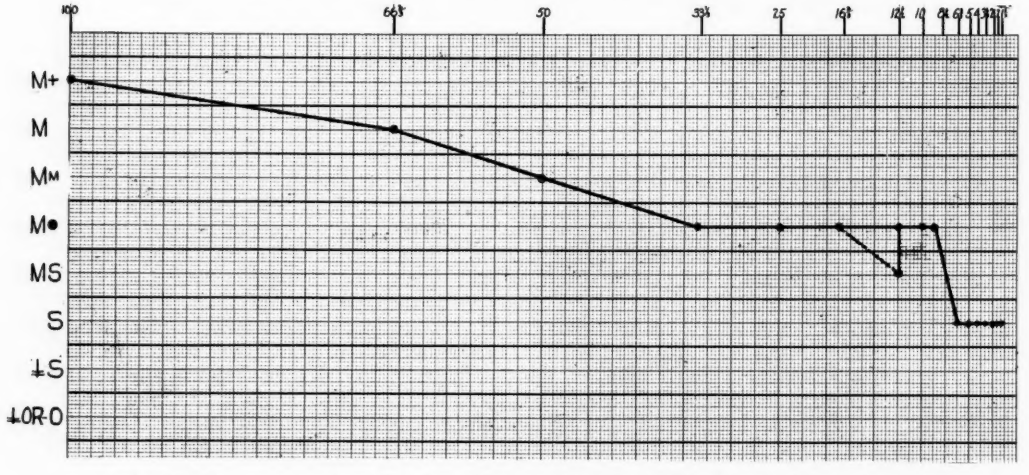

Fig. 8.—Extracts weaker in concentration than the original by 33⅓ per cent by 50 per cent and 66⅔ produce progressively smaller reactions, but a plateau of similar sized reactions occurs between 33⅓ and 8½, a diminution in concentration of 74 per cent. Note the different reactions produced by 12½. Between 8½ and 6⅔, there is a very distinct difference in reaction size, but between 6⅔ and 1⅔ is a plateau, although the diminution in concentration is 75 per cent. If the lower instead of the upper curve is read, the first plateau extends between 33⅓ and 16⅔.

were obtained. The reaction curve, using the scratch test, was completed in 17 cases.

Analysis of the skin reaction curves with this test with 3 per cent Bermuda grass pollen extract in serial dilution, shows characteristics

similar to those seen with house dust extract. Here, again, a discontinuous curve is obtained, with plateaus of similar sized reactions occurring in all ranges of concentration of the extract, and with all sizes of reactions.

B. Intracutaneous Tests.—A 3 per cent Bermuda grass extract was also used for these tests. The following dilutions were made by the

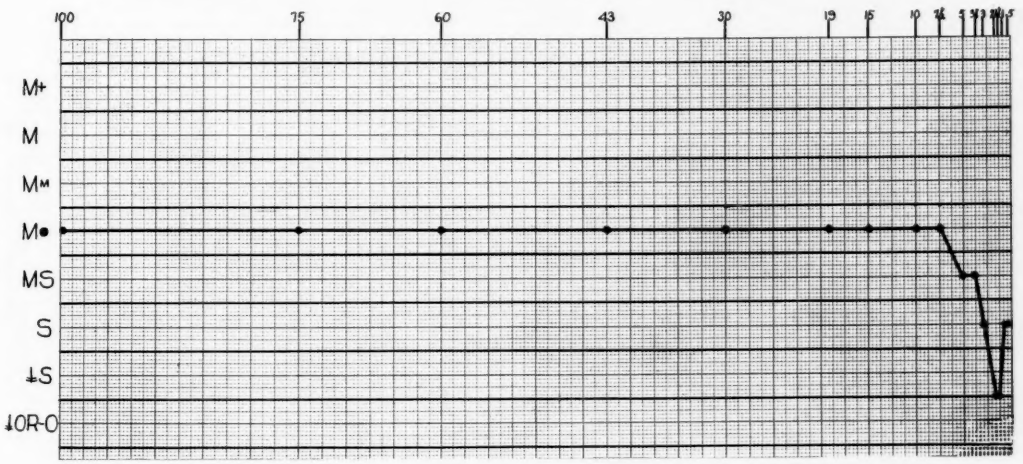

Fig. 9.—Plateau of equal sized reactions between 11 and 7½, a diminution in concentration of 92 per cent; then a rapid fall in the reaction size between 7½ and 2. Note particularly the larger reaction at 0.5 than the reaction at 1.

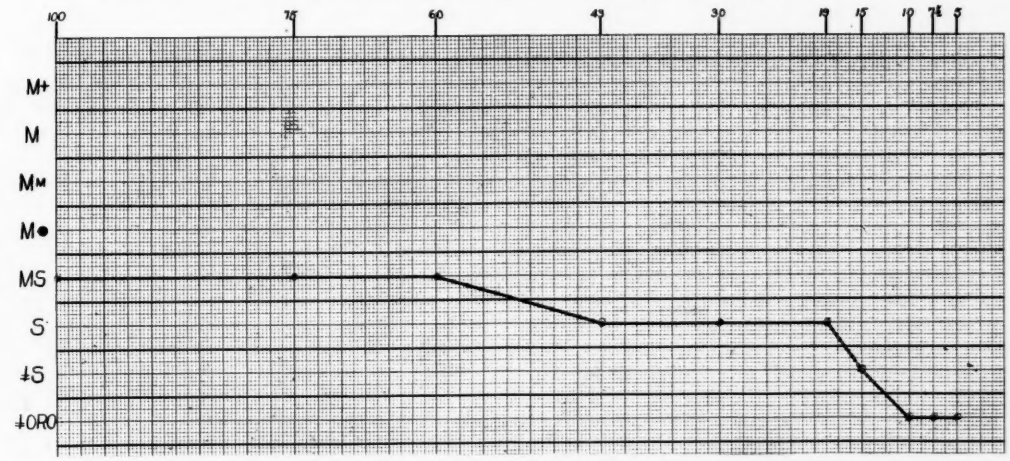

Fig. 10.—Plateau between 100 and 60, a concentration diminution of 40 per cent; another plateau between 43 and 19 (43 is about 2¼ times as concentrated as 19); and a difference when the concentration is diminished by 22 per cent; and again by 33 per cent.

addition of Coca's solution: $\frac{Gr.}{15}$, $\frac{Gr.}{20}$, $\frac{Gr.}{25}$, $\frac{Gr.}{35}$, $\frac{Gr.}{50}$, $\frac{Gr.}{75}$, $\frac{Gr.}{100}$, $\frac{Gr.}{150}$, $\frac{Gr.}{200}$, $\frac{Gr.}{300}$, $\frac{Gr.}{400}$, $\frac{Gr.}{500}$, $\frac{Gr.}{750}$, $\frac{Gr.}{1000}$, $\frac{Gr.}{1500}$, and $\frac{Gr.}{3000}$. The $\frac{Gr.}{15}$ dilution (0.2 per cent

extract) instead of the original 3 per cent extract was used as the base of 100 for calculation of percentage concentrations.

The reaction curves* for the grass extract with the intracutaneous

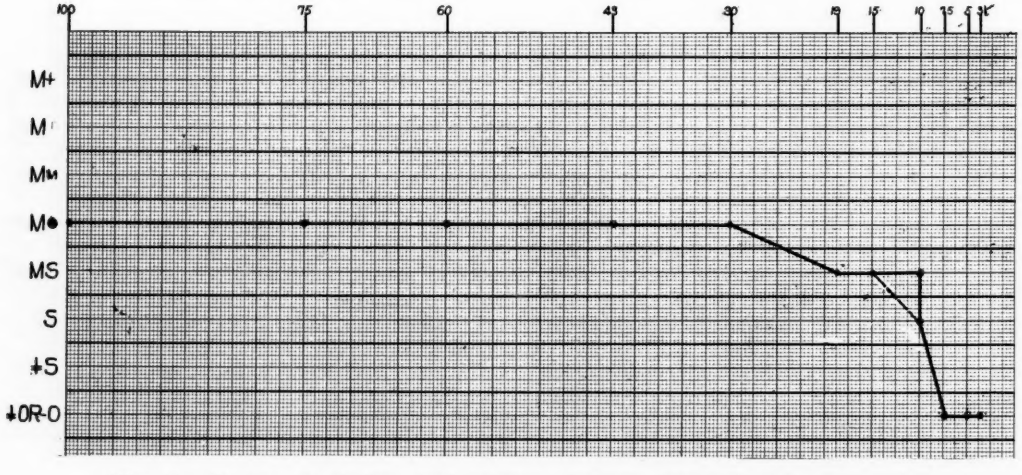

Fig. 11.—Plateau of similar sized reactions between 100 and 30, a 70 per cent diminution in concentration; between 30 and 19 a difference in the reaction sizes; another plateau between 19 and 10 (for the upper curve, or between 19 and 15 for the lower). Note the different reactions for the same extract concentration at 10, and also, the rather rapid disappearance of positive reactions.

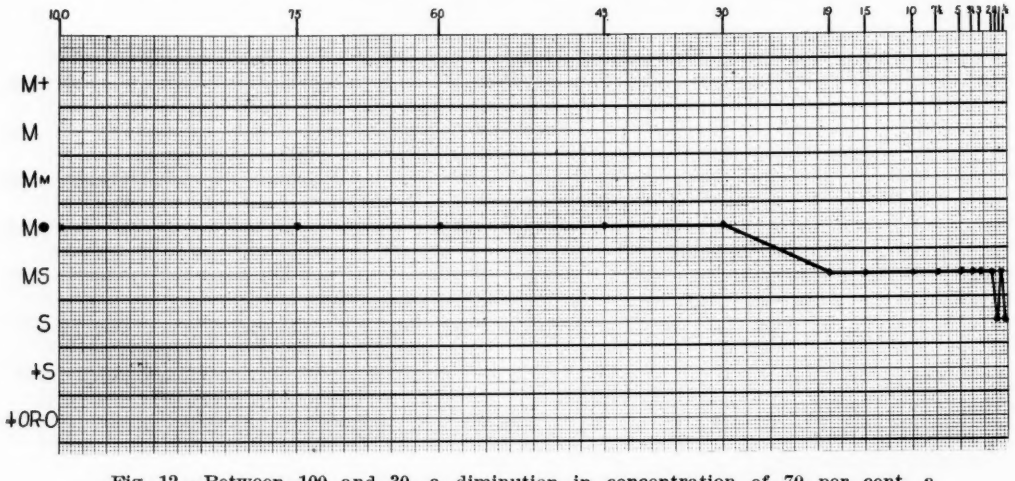

Fig. 12.—Between 100 and 30, a diminution in concentration of 70 per cent, a plateau of similar sized reactions occurs; another plateau occurs between 19 and 2, a concentration diminution of 89 per cent. Note the larger reaction at 1 than at 1½.

technique is represented by Figs. 9, 10, 11, 12, 13, and 14. No graphs essentially different from those presented were obtained. The reaction curve was completed on 15 cases.

*Wheals with pseudopods: *M+*, very large wheals, marked erythema; *M*, large wheal, marked erythema; and *Mm*, wheal, marked erythema. Wheals without pseudopods—*Mo*, wheal 2 to 3 times the size of control; definite erythema; *Ms*, wheal definitely larger than control, definite erythema; *S*, wheal and erythema slightly larger than control; ±*S*, wheal and erythema questionably larger than control; and ± or 0, wheal and erythema same as control.

Analysis of the curves obtained with the intracutaneous tests with serial dilutions of a 3 per cent Bermuda grass extract, shows the same discontinuity observed with the scratch tests. The plateaus have a tendency to spread over a greater range of difference in concentration

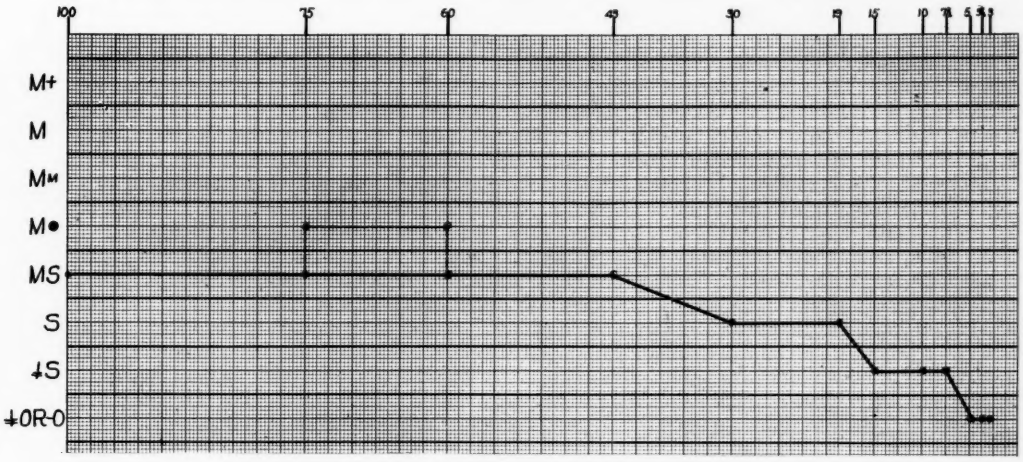

Fig. 13.—Note particularly the different sized reactions obtained for identical concentrations at 75 and 60; this is a not infrequent finding with the intracutaneous tests. The curve is characteristically discontinuous.

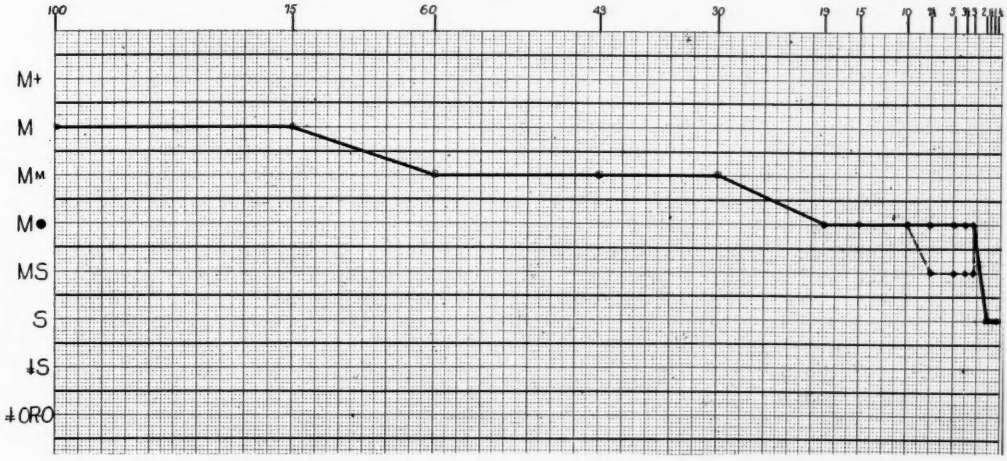

Fig. 14.—A plateau between 100 and 75; another plateau between 60 and 30, and another between 19 and 3 (a concentration diminution of 84 per cent if the upper curve is read); or between 19 and 10 if the lower curve is read (a diminution in concentration of 47 per cent). Note unequal reactions for the same concentrations between 7 and 3. This finding not infrequent with intracutaneous tests. Another plateau between 5 and 2.

with this technique than with the scratch tests. Plateaus occur in all ranges of concentration of the extract and with all sizes of reactions. Extracts of lesser concentration producing larger reactions than extracts of greater concentration were more frequently found with this type of test than with the dermal test.

DISCUSSION

The skin reaction curve obtained with serial dilutions of house dust and Bermuda grass extract is a discontinuous one, so that in a given subject, a given size skin reaction can be produced by potencies which vary tremendously. Equal sized skin reactions may denote a very wide range of difference in potency. There is an overwhelming tendency toward similar sized reactions in the upper ranges of concentration of the extract. However, plateaus occur at all ranges of concentration and with all sizes of reactions. The discontinuity of the skin reaction curve is obtained despite the presence of factors which affect the size of the skin reactions, and which would tend to produce unequal reactions even if extracts of the same concentration and amount were used.

While the work presented here was done on only house dust and Bermuda grass pollen extract, it is probably predicable that the same type curve will be found with other extracts. This, of course, will have to be determined.

CONCLUSIONS

1. Skin reaction curves to house dust and grass pollen extracts have been described.

2. These curves are discontinuous, with plateaus in all ranges of concentration of the extracts, but especially in the range of higher concentrations.

3. Extreme caution must be taken in applying comparative skin tests to the quantitative evaluation of antigenic activity.

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HYPERSENSITIVENESS TO TOBACCO AND BIOPSY STUDIES OF SKIN REACTIONS IN VASCULAR DISEASE*†

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IN PREVIOUS publications we reported the findings of positive skin reactions and reagins to tobacco in patients suffering from thromboangiitis obliterans, certain types of coronary artery disease and migrating phlebitis.[1-4] Most of these patients, especially those with thromboangiitis obliterans and migrating phlebitis did not present evidence of the commoner allergic diseases, such as asthma or hay fever, which would allocate them to the constitutionally allergic category. It was deemed necessary, therefore, for further identification, to determine whether the tissue reactions following the intradermal injection of tobacco in these patients were histologically similar to those elicited by allergens injected into the constitutionally allergic patients as described by Berger and Lang,[5, 6] Klein, Cohen, and Rudolph[7] and others.

According to these authors, particularly Klein, Cohen, and Rudolph the urticarial wheal following the intradermal injection of an allergen or histamine into the allergic patient is characterized microscopically by an eosinophilic inflammatory exudate which reaches its height within one-half hour, and then gradually recedes. No such reaction to an allergen or histamine is obtained in the so-called normal or nonallergic individual. In agreement with Berger and Lang, Klein, Cohen, and Rudolph conclude that eosinophilia in the inflammatory exudate, while not constant or essential, is nevertheless the most characteristic sign of allergic reactions.

To determine the nature of the tissue response in the patients with vascular disease we made histologic studies of biopsies of skin reactions resulting from the intracutaneous injections of tobacco extracts and wheals produced by saline and ragweed extracts used as controls in fifteen individuals. Ten of these suffered from thromboangiitis obliterans, one had migrating phlebitis and one coronary artery disease. Three surgical patients were used as normal controls.

Each of these cases was tested simultaneously with six different types of tobacco as well as ragweed, timothy, horse dander extracts, and Coca's diluting fluid. Five of the tobacco extracts used were made from the cured leaves of Burley, Maryland, Virginia, Xanthis, and

*From the Medical Department and Laboratories, Mount Sinai Hospital, New York.
†Read before the Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., May, 1937.

TABLE I

	CASE	AGE OF ONSET	PRESENT AGE	TOBACCOS TESTED: BURLEY	COCA	MARYLAND	VIRGINIA	XANTHIS	HAVANA	DILUENT	RAGWEED	TIMOTHY	HORSE DANDER	TOBACCO PATCH TEST	PASSIVE TRANSFER TC TOBACCO	PASSIVE TRANSFER TC POLLEN	TOBACCO BIOPSY EOSINOPHILIA	SALINE SITE	RAGWEED SITE	HISTORY OF ALLERGY
									Thromboangiitis Obliterans											
1	H. B.	43	44	+	+	+	+	+	0	0	0	0	0		Pos.		+++*	0		0
2	W. P.	33	42	0	+	0	0	0	0	0	0	0	0		Pos.		+++	0		0
3	E. R.	38	45	++	+	++	++	++	++	0	0	+	+		Pos.	Neg.	++	0		0
4	A. F.	39	40	+	±	+	++	++	++	0	0	0	0		Pos.		+	0		0
5	Y. T.	39	41	++	0	+	+	+	++	0	0	0	0				++	0		0
6	P. C.	38	39	+	0	±	±	+	0	0	0	0	0		Neg.		+	0		0
7	H. L.	37	38	0	0	0	0	+	0	0	0	0	0		Neg.		0	0	0	0
8	J. A.	20	37	++	++	++	0	±	++	0	0	0	0		Neg.		0	0		0
9	W. W.	33	45	0	D†	0	0	0	D	0	0	0	0	0	Neg.		Derm. +‡	0		Hives
10	R. G.		23	+D¶	0	+D	±	0	0	0	0	0	0				Derm. +	0		0
									Migrating Phlebitis											
11	S. C.		40	+	++	+	0	0	++	0	+++	+	0		Pos.		+++	0		0
									Coronary Artery Thrombosis											
12	R. E.		37	0	D	0	D	0	0	D	0	0	0	0			Derm.	0		0
									Controls											
13	A. G.		20	+	0	+	+	+	0	0	++	++	0				0	0	Eos. +++	H. F.§
14	J. D.		38	0	0	0	0	0	0	0	0	0	0				0	0	0	0
15	L. S.		42	0	0	0	0	0	0	0	0	0	0				0	0	0	0

*+++ Marked Eosinophiles
++ Moderate Eosinophiles
+ Few Eosinophiles
0 No Eosinophiles

†Delayed Reaction
‡Derm. = Dermatitis
¶Delayed reaction followed immediate "+" reaction
§H.F. = Hay fever

Havana tobaccos, the sixth was a commercial extract composed of a mixture of tobaccos prepared by Lederle, which we designated as Coca extract. The intradermal test dose was 0.01 to 0.02 c.c.

Positive skin reactions to tobacco were obtained in the 12 patients under investigation and in one of the 3 control cases.

The type of reaction was characterized by an immediate urticarial wheal in 10* of the patients and a delayed type of reaction in 2. Of the 3 controls, one showed an immediate reaction to tobacco and ragweed, the other 2 were completely negative. Of the 10 who gave immediate reactions, 5 had reagins to tobacco on passive transfer.

Every one of the cases of thromboangiitis obliterans presented typical features of this disease. Each gave a history of onset of vascular involvement before the age of 45. In this, as in all our previous publications on the incidence of positive skin reactions to tobacco in thromboangiitis obliterans, we were especially careful in reporting only those cases which conformed to every criterion generally accepted as diagnostic for this particular vascular involvement. Each patient was carefully studied by Dr. S. Silbert and staff. Young individuals with arterial disease due to infections or various toxic agents, presenile arteriosclerosis associated with diabetes as well as those developing vascular disease between the ages of 45 to 55 were excluded. We regard the latter as late, or borderline cases of thromboangiitis obliterans, who, while presenting a picture of thromboangiitis obliterans, may be suffering from early arteriosclerosis or may possibly have both conditions. The differentiation of these two groups is important from the point of view of etiology as well as prognosis. In the typical cases of thromboangiitis obliterans developing under 45, tobacco plays an essential rôle. We have found that approximately 70 per cent of these patients show positive skin reactions of one-plus or greater to one or more types of tobacco used in our tests, and almost 45 per cent have tobacco reagins in their blood serum, demonstrable by means of passive transfer. Elimination of tobacco arrests the progress of this disease, resumption of smoking induces an exacerbation of all symptoms. The prognosis in this group is relatively good and is basically dependent on the cessation of the use of tobacco. The borderline cases of thromboangiitis obliterans which may present complications, such as early arteriosclerosis, may be improved symptomatically by the removal of tobacco. However, because of the very nature of the underlying vascular degeneration, the control of the disease may not be as readily accomplished.

A summary of the patients who comprise this report and the manner in which they reacted are illustrated in Table I.†

*Case 10 had both immediate and delayed reaction.

†The biopsies in each case were examined by Dr. Samuel Peck of the dermatologic staff and assistant dermatopathologist of Mount Sinai Hospital.

The patients under investigation were first skin tested with the usual technique indicated above. After recording the results of these tests, they were asked to return the next day for the purpose of obtaining a biopsy of the tobacco-skin reaction. In order to obtain a large enough area of skin for histologic study we injected 0.05 c.c. of the test extract instead of 0.01 to 0.02, as routinely employed in our diagnostic tests. Each control site whether injected with saline or ragweed also received 0.05 c.c. We found that this amount had no influence on the results. The originally negative reaction resulting from the injection of 0.01 c.c. of diagnostic extract remained negative after 0.05 c.c. was introduced, except that the wheal induced by this quantity of material was somewhat larger. Thus two of our cases of thromboangiitis obliterans, who gave positive reactions to tobacco with ordinary diagnostic tests, failed to show an eosinophilic type of response histologically when 0.05 c.c. of tobacco extract or buffered saline was used, indicating that this larger volume of injected material had no influence on the character of the cellular response. All of our control cases likewise showed negative tissue reactions following the injection of this amount of test extract, and each saline control wheal consisting of 0.5 c.c. of Coca's buffered salt solution was negative in every instance. The skin section to be studied was excised approximately one-half hour after the intradermal injection, which was usually made in the outer part of the upper arm. The area surrounding the urticarial wheal was anesthetized with 2 per cent novocaine. The segment removed was about one-half inch to three-fourths of an inch in diameter and contained the urticarial wheal. This was fixed in 10 per cent formalin and stained with hematoxylin and eosin.*

The protocols of Cases 1, 9, 11, and 12 are herewith reported in full. Inasmuch as the course and physical signs of the other 8 cases of thromboangiitis obliterans are essentially similar, with exception of variations in degree and limbs involved, we are confining ourselves to descriptions of patients who illustrate significant points at issue. The results of skin reactions, passive transfer, and histology of the biopsy sections of all the cases are completely recorded in Table I.

CASE REPORTS

THROMBOANGIITIS OBLITERANS

CASE 1.—H. B., aged 44 years, was first seen on August 18, 1934, complaining of inability to walk more than two blocks without severe pain in the calf of the left leg. He also complained of pain and swelling of the left big toe. There was no history of asthma, hay fever, or other allergic disease. He had smoked about 20 cigarettes daily as long as he could remember.

*The biopsies were excised by Dr. Robert Abrahamson.

Physical examination of the heart, lungs, and abdomen was negative. Fluoroscopy of the chest was negative. His blood pressure was systolic 150, diastolic 80. Radial pulses were normal with no evidence of arteriosclerosis. The electrocardiogram was normal. Lower extremities, left big toe and adjoining part of the left foot, were in a state of rubor. No pulse was felt. The right anterior tibial artery was open but no pulse was felt over the right external malleolus. The oscillometric reading at the right ankle was 4.0, with only a very faint oscillation at the left ankle. Diagnosis, thromboangiitis obliterans.

The patient was told to stop smoking and was treated with intravenous hypertonic saline solution. In October, 1934, the left big toe became gangrenous and underwent spontaneous amputation. Saline treatment was continued for the subsequent two years. On May 9, 1935, x-ray of the left foot showed evidence of amputation of big toe through the shaft of the proximal phalanx. There was a very marked diffuse spotty atrophy of the entire foot. No evidence of osteomyelitis. The atrophy of the foot indicated that the vascular damage in this patient noted clinically on admission had been fairly extensive. In spite of this there was improve-

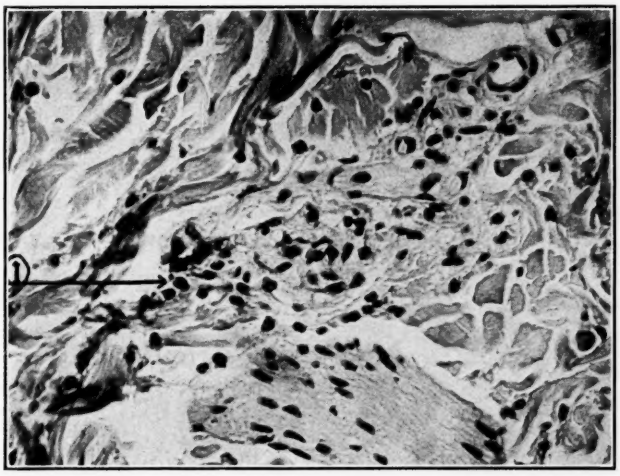


Fig. 1.—Case 1. High power photomicrograph. *1*, Tobacco injected site. Dilated capillaries showing perivascular infiltrations of leucocytes and eosinophiles.

ment in the circulation according to the oscillometric reading. In February, 1935, it was 0.25 at the left ankle, whereas on admission the reading could scarcely be recorded.

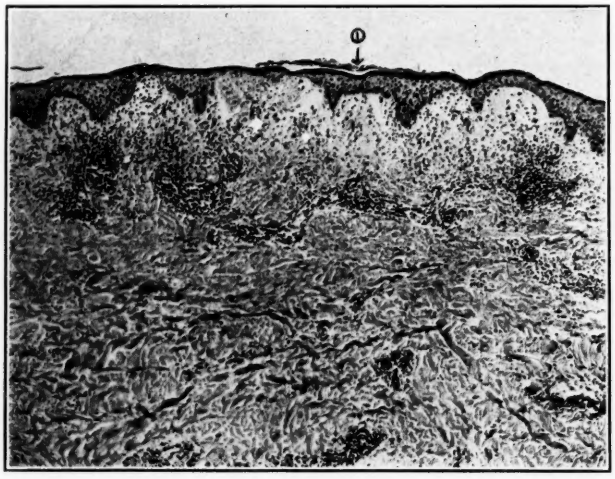
On January 25, 1936, the patient felt quite well. The ulcer at the base of the left big toe was almost healed. While no pulse was felt at the ankle by palpation, the oscillometric reading was definitely better. On the right foot pulses were felt at the right anterior tibial region and also at the external malleolus.

Skin tests showed positive reactions to all tobaccos (see Table I). Passive transfers to tobacco were also obtained.

Biopsy Report of Skin Sections.—The saline control section is essentially negative, except for some edema in the upper cutis and a number of dilated capillaries. There is some perivascular edema in the upper and middle cutis. In the tobacco injected area, in and about the subcutis, but most marked in the middle cutis, there are many dilated capillaries showing perivascular infiltration of leucocytes and many eosinophiles. Throughout the section are scattered leucocytes and many eosinophiles.

CASE 9.—W. W., aged forty-five years, was admitted October, 1935. His chief complaint was pain in the left leg for twelve years. His present symptoms began in

1917, when he experienced difficulty in walking. In December, 1934, he developed an ulcer on the left big toe. He was treated in the Veterans Hospital, where he was told to stop smoking. He had smoked 60 cigarettes daily since the age of 16. There was no family history of allergy. He had hives at one time, years ago. He frequently suffers from headaches.



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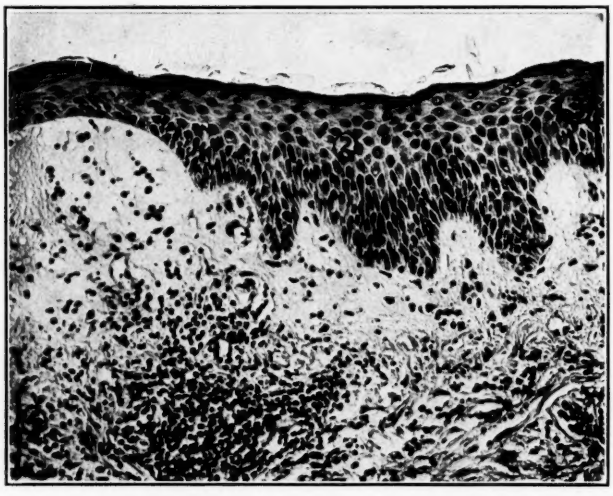


B.

Fig. 2.—Case 9. *A*, Low power photomicrograph of the eczematous lesion. *1*, Parakeratotic areas.

B, High power photomicrograph of the eczematous lesion shown above. *2*, Spongiosis = Intercellular edema. *3*, Acanthosis. *4*, Acute inflammatory reaction, edema, polymorphonuclear cells and lymphocytes.

Physical examination of heart and lungs was negative. Blood pressure was systolic 130, diastolic 90. Both femoral arteries were open. The right popliteal was open, the left closed, the right posterior tibial artery was open, the right anterior tibial was closed. No pulsation was felt in left foot. Oscillometric reading: right ankle 3, left 0.5.

Skin tests for tobacco gave negative immediate reactions. Three to four days later, delayed reactions, eczematoid in character, appeared at the tested sites injected with Coca and Havana tobaccos. No passive transfer tests to tobacco were obtained. The patch test to tobacco was negative. An attempt at desensitization with tobacco extracts was followed by a generalized eczema of hands and face, similar in appearance to the delayed skin reaction. Simultaneously, pains developed in the legs just as on admission. The patient had frequent headaches and became markedly constipated. The tobacco injections had to be stopped. Following this, the eczema disappeared, the pain in legs subsided, and the patient felt well again.

Biopsy Report of Skin Sections.—Microscopic section of the delayed reaction shows the following: scattered parakeratotic areas; moderate acanthosis. In places where there is parakeratosis, especially the lower layers of the epidermis, there is intracellular edema and some spongiosis. Papillary and subpapillary areas below the edematous epidermal portions just described show an acute inflammatory reaction, edema, polymorphonuclear cells, and lymphocytes. In the middle cutis there are many dilated vessels with large perivascular infiltrations, consisting mainly of round cells and some polymorphonuclear cells as well as a very few eosinophiles (Fig. 2).

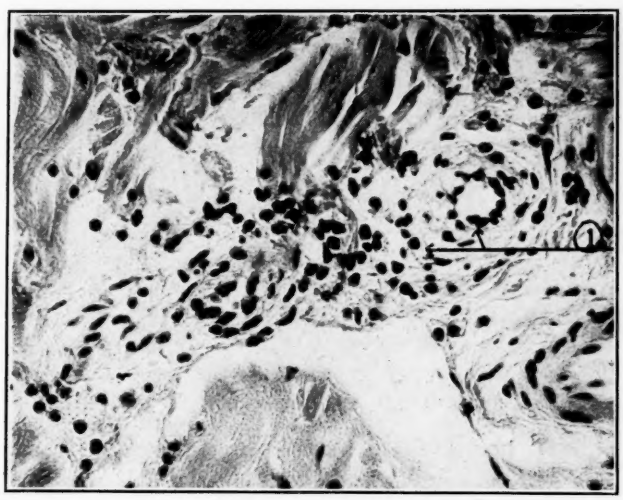


Fig. 3.—Case 11. High power photomicrograph. *1*, Marked perivascular infiltration and invasion of the walls of arterioles with eosinophiles.

MIGRATING PHLEBITIS

Case 11.—S. C., aged 40 years, was admitted April 23, 1934, to the vascular clinic, complaining of the fact that each summer he gets red blotches on his legs, which he thinks are due to something he eats. There was no personal nor family history of allergy. He had been smoking heavily for many years.

Physical examination of the heart and lungs was negative. Examination of both legs showed areas of segmental phlebitis. Phlebitic areas were also present over lower anterior left thigh. The arterial circulation was normal in both legs. His phlebitis disappeared after cessation of smoking. During the next two years of observation, he had one recurrence after he resumed smoking. Skin tests showed positive skin reactions to four out of six tobaccos tested. He also reacted to pollens, feathers, pyrethrum, orris root, etc. Passive transfer tests were obtained to tobacco. Tests to various fruits were negative.

Biopsy Report of Skin Sections.—Saline control wheal is negative. The tobacco urticarial wheal shows the epidermis thin but not especially atrophic. There is fat replacement of the cutis in spots. Below the epidermis and toward the middle layer of the cutis there are many thin-walled vessels markedly engorged and dilated. They show a very marked perivascular infiltration and even invasion of the walls of an acute type, made up mostly of eosinophiles. Some of the small arterioles show leucocytic infiltration of the walls. There are no special changes seen in the endothelium lining the vessels. A number of hair follicles, as well as the sebaceous glands, show polynuclear infiltrations made up of leucocytic mononuclear cells and many eosinophiles.

CORONARY ARTERY THROMBOSIS

CASE 12.—R. E., aged 37 years, was admitted to the cardiac clinic November 10, 1936. His occupation was that of an instructor in physical education. He complained that five days before coming to the clinic he experienced precordial pain radiating down the left arm and fingers. His past history was negative for diabetes or hypertension. He had smoked 20 to 30 cigarettes a day. He stated that during the past year he had repeated attacks of precordial pain whenever he smoked. He stopped for a while but began again. This was followed by a recurrence of severe precordial pain which brought him to the hospital.

Physical examination disclosed a well-developed individual, who did not look very sick. Lungs were normal. The heart sounds were of good quality, first sound at the apex was split; A_2 louder than P_2. No murmurs were heard. Blood pressure was systolic 130, diastolic 90. Venous pressure was 6.3 cm. Saccharin circulation time was thirteen seconds. The electrocardiogram on November 10 indicated sinus bradycardia, rate 60; left ventricular preponderance; T_2 diphasic; T_3 inverted. A second electrocardiogram, taken four days later, showed progressive changes of coronary thrombosis. The white blood cell count was 19,850 and polymorphonuclear cells 50 per cent. The Wassermann test was negative.

Skin tests to tobacco showed delayed reactions appearing three to four days after injection to Maryland, Virginia, and Havana tobaccos. Patch test to tobacco was negative. No passive transfer was obtained.

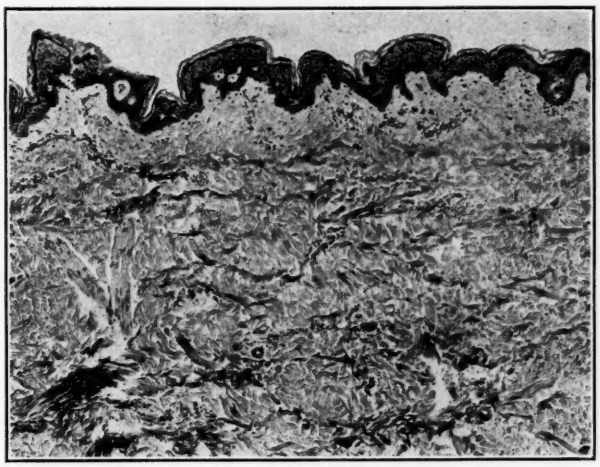
Biopsy Report of Skin Sections.—The epidermis of the tobacco-injected skin segment shows parakeratotic areas and spongiosis (Figs. 4*B* and *C*). The papillary and subpapillary bodies show marked inflammatory reaction consisting of dilated engorged capillaries, marked perivascular infiltration, consisting mainly of mononuclear cells, some polynuclear, few plasma cells, and no eosinophiles. The picture is that of an eczema. The saline control wheal is negative (Fig. 4*A*).

NORMAL CONTROLS

Three surgical cases, who consented to biopsies, were tested with tobacco, ragweed, timothy, and horse dander extracts. One was a case of fissure in ano and ragweed hay fever; the second, inguinal hernia; and the third had chronic cholecystitis.

CASE 1.—A. G.—Diagnosis: fissure in ano. This patient reacted to ragweed and tobacco extract on intradermal tests.

Biopsy Report of Skin Sections.—The immediate urticarial wheals produced by ragweed and tobacco showed the following: ragweed section shows a normal epidermis; midcutis shows many dilated vessels with perivascular leucocytes, many of which are eosinophiles. The tobacco sections show a normal epidermis. Midcutis shows some dilated vessels, less vascular infiltration than the ragweed section. The perivascular infiltration consists of polymorphonuclear cells with practically no eosinophiles. Saline control showed normal skin.



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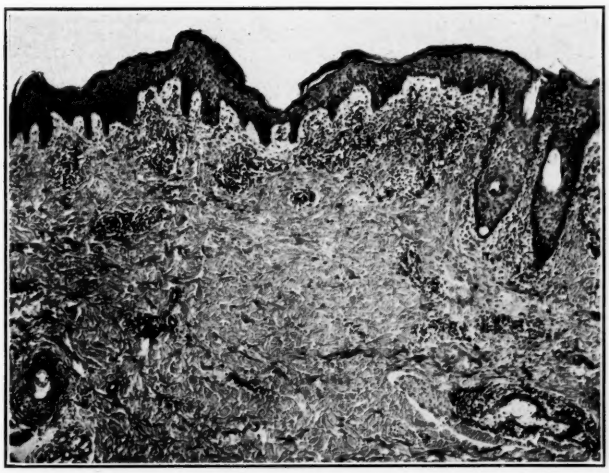

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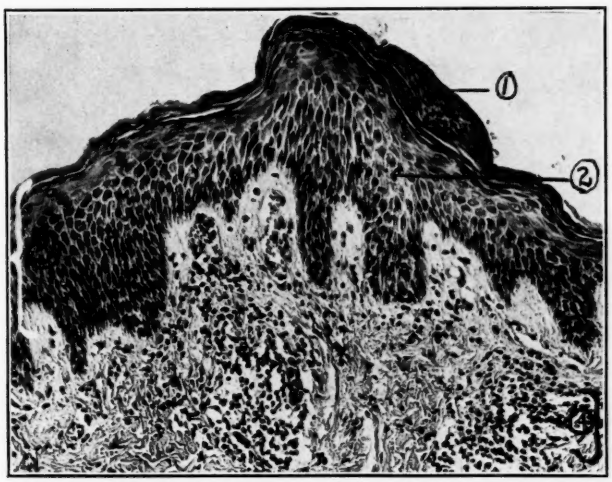

C.

Fig. 4*A*, *B*, *C*.—Case 12. *A*, Low power photomicrograph of negative saline control wheal. *B*, Low power photomicrograph. Eczematous type of delayed skin reaction to tobacco. *C*, High power photomicrograph. *1*, Parakeratosis. *2*, Spongiosis = Intercellular edema. *3*, Acanthosis. *4*, Inflammatory reaction consisting of perivascular infiltration of mononuclear cells, polymorphonuclear and plasma cells.

CASE 2.—J. D.—Diagnosis: inguinal hernia. Skin tests to tobacco, ragweed, timothy, and horse dander were negative.

Biopsy Report of Skin Sections.—The negative skin sites to tobacco and ragweed show the following: ragweed section presents atrophic epidermis, upper cutis and midcutis showing a moderate number of dilated vessels, with some perivascular infiltration consisting of lymphocytes and some polymorphonuclear cells. No eosinophiles are seen. The tobacco section has practically the same appearance. No eosinophiles are seen.

CASE 3.—L. S.—Diagnosis: chronic cholecystitis. Skin tests to tobacco, ragweed, timothy, and horse dander were negative.

Biopsy Report of Skin Sections.—Tobacco section shows some dilated vessels in upper cutis with scattered perivascular infiltration. Saline control section shows some perivascular inflammatory reactions and nothing else.

DISCUSSION

A review of the findings in the 12 cases with vascular disease and 3 normal controls, all smokers, tested with six different tobaccos, diluent, ragweed, timothy, and horse dander extracts indicates the following facts:

None of the 12 patients with vascular disease included in this study had any personal or family history of allergy. This, however, may be only accidental, inasmuch as in a larger series of cases, consisting of 140* patients with thromboangiitis obliterans and 100 with coronary artery disease, there was a total incidence of 25 per cent of individuals with family and personal histories of allergy. This is comparable to similar findings in 200 unselected normal control smokers, investigated from the same points of view.[4] In contrast, however, to the patients with thromboangiitis obliterans where a history of allergy was obtained in only 10 to 12 per cent of cases, such positive histories were elicited in 33 per cent of young patients with coronary artery syndrome. The exact significance of this difference is not clear, except that one might infer that the factor of inherited or so-called atopic hypersensitiveness is not as prominent in the group suffering with thromboangiitis obliterans. Nevertheless, sensitization to tobacco in the wider allergic sense, which we have repeatedly stressed, has been demonstrated by us to be present in 70 per cent of typical cases of thromboangiitis obliterans. If we were to subtract from this number the 10 per cent giving histories of allergy and multiple reactions to various combinations of ragweed, timothy, horse dander, and tobacco, we would have 60 per cent giving reactions to tobacco only.

The criteria for identification of the allergic mechanism, such as positive skin reactions, demonstration of specific reagins, elicitation of symptoms by the specifically reacting antigen, countered by their disappearance on withdrawal of the offending agent, have been adhered to throughout our work.

*Unpublished figures.

In the present series we found that 8 cases with thromboangiitis obliterans and the one with migrating phlebitis, gave immediate urticarial reactions to one or more of the tobacco extracts. Of these 9 patients, 5, or 55 per cent, had demonstrable reagins to tobacco on passive transfer. This is approximately in accord with our larger, as yet unpublished, series of 140 cases, in which 45 per cent showed tobacco reagins. The remaining two cases of thromboangiitis obliterans (9 and 10) as well as the 37-year-old patient with coronary artery thrombosis (Case 12), developed delayed skin reactions two to four days after intradermal injections of tobacco extract. Such reactions were characterized by an acute dermatitis, localized to the injected site. The delayed reactions to Burley and Maryland tobaccos, which appeared in Case 10 on the third day, were preceded by immediate urticarial (one-plus) reactions to these same tobaccos. This transition from an urticarial to an eczematous type of reaction is significant in that it indicates that the same antigen can evoke two types of reactions in a sufficiently hypersensitive individual.

Patch tests with various tobaccos in Cases 9 and 12 which had given delayed reactions, yielded negative results. This indicated that the dermatitis was not in the nature of a contact reaction.

None of these three patients had any tobacco reagins which could be demonstrated on passive transfer. Yet the etiologic specificity of the tobacco reaction was strikingly emphasized in Case 9 (W. W.), suffering with thromboangiitis obliterans when we attempted to desensitize him with Havana tobacco to which he gave a delayed reaction. After fifteen inoculations with 0.1 c.c. of Havana tobacco extract, at intervals of five days, a generalized dermatitis ensued involving the face and hands similar in appearance to the delayed reactions which had developed after the intradermal tests. Pains in both legs (intermittent claudication) difficulty in walking, headaches, and severe constipation which had caused him to seek medical advice, also recurred. The tobacco injections had to be stopped, following which all his symptoms as well as the dermatitis disappeared.

The deliberate precipitation of the characteristic symptoms of thromboangiitis obliterans, as well as other systemic manifestations consequent upon the introduction of tobacco by the subcutaneous route in this case, signalizes an unpremeditated reproduction of the clinical picture, similar in every respect to that repeatedly observed in our patients following the routine use of tobacco.

While it may be argued that the intermittent claudication, headache, and constipation are subjective phenomena, no one can deny the factual evidence supplied by the outbreak of the generalized dermatitis *pari passu* with the recrudescence of the vascular symptoms, or the simultaneous disappearance of both, following the stopping of tobacco injections.

The artificially superinduced effect in this patient is supplemented by numerous analogous clinical experiences with tobacco of many workers in the field of peripheral vascular disease. In recent cases of thromboangiitis obliterans, Silbert[8] reports complete arrest in the progress of this disease following cessation of smoking. This of course does not take into consideration cases in which agents other than tobacco are responsible for a vascular disease picture simulating thromboangiitis obliterans, the very advanced cases where long-standing organized, irreversible thrombosis exists, or patients in whom complications of arteriosclerosis are present. In addition to cessation of the progress of the disease in the early cases, we have repeatedly noted prompt reappearance of symptoms on resumption of the use of tobacco. In the 10 cases of thromboangiitis obliterans and one of migrating phlebitis comprising this report, the discontinuance in the use of tobacco had a distinct bearing on the control of symptoms and progress of the disease.

Observations of a nature similar to those illustrated in Case 12, of this group, with coronary thrombosis and angina pectoris associated with the use of tobacco, have also been reported by us in a group of relatively young individuals sensitive to tobacco. While excessive smoking was an outstanding feature associated with symptoms of angina pectoris in many of our young patients, there were a fair number among them who smoked in moderation, but nevertheless were clinically affected by tobacco. These patients gave positive skin reactions and had demonstrable reagins in their serum corresponding to the tobacco allergen. Neither the degree of skin reactivity, however, nor the presence or absence of reagins, necessarily paralleled the clinical severity of the vascular disease in question. As indicated in Table I, although patients 5, 7, and 8 suffering with thromboangiitis obliterans, gave one- and two-plus reactions on direct tests, no reagins to tobacco could be demonstrated on passive transfer. Nevertheless, the clinical course of the disease differed in no way from that observed in the other patients in this group in whom tobacco antibodies were present. Such variations in immunologic response are to be found not only in the group of patients under discussion, but likewise in all other well recognized allergic diseases. This has been pointed out by Harkavy and Witebsky[9] in a previous publication, and recently stressed by Chobot and Hurwitz.[10]

In addition to a lack of correlation between the clinical course of the disease and the immune reactions, biopsy studies of the tobacco-injected sites in our cases with vascular disease likewise disclosed varying degrees of eosinophilic response. Eosinophilia differing in intensity was found in the biopsy sections of 7 of 12 cases. In Cases

7 and 8 no tissue eosinophilia appeared, although both of these patients gave positive skin reactions to tobacco. In Cases 9, 10, and 12, the allergic nature of the tobacco test was apparent in the eczematoid character of the delayed reaction which appeared three to four days after intradermal injection of the specific antigen. This remained for two to three weeks. The specificity of this response, as has been pointed out, was borne out by the fact that repeated injections of tobacco extract produced not only a generalized eczema in Case 9, but also an exacerbation of the thromboangiitis syndrome. No such local or general response followed the introduction of ragweed, timothy, and horse dander extract in any of our cases. No such reactions ever occurred in any of the 200 normal controls tested with tobacco, who were not sensitive to it. No eosinophilic tissue response in the tobacco wheal was observed in the biopsies of 3 surgical control cases insensitive to tobacco.

These findings serve to emphasize that the tobacco extract, far from being a primary urticariogenic substance like histamine or other drugs, is an allergen capable of evoking in susceptible individuals both immediate and delayed reactions, the specificity of which is substantiated by the presence of serum reagins and local eosinophilic tissue response. The fact that all three types of reactions do not always appear in any one individual simply means that the immune mechanism in such a person is limited. It does not nullify the antigenicity or specificity of the tobacco protein.

The finding of such immune reactions per se, however, does not imply etiologic significance. As has been repeatedly stressed, the etiologic nature of an antigen in a disease where an allergic mechanism is suspected must be established not only by the demonstrations of immune reactions, but also by the ability of such an antigen to evoke the clinical symptoms of the disease on contact and their disappearance on removal. This has been shown clinically to be a fact in the cases presented herewith and clearly proved in Case 9. Yet this patient, whose symptoms were evoked by tobacco injections and stopped by their withdrawal, gave a delayed eczematous type of skin reaction and had no circulating antibodies to tobacco. Such a discrepancy serves to emphasize the fact that tissue and organ susceptibility are separate and distinct from other manifestations of immunologic reactivity. Neither the antibody content of the serum nor skin responses alone are an exact mirror of the state of the shock organ. This has been clearly demonstrated by Teale[11] in his experimental work dealing with the rôles played by the tissues and circulating antibody in immunity. Correlation of all the known facts, clinical and laboratory, is obviously necessary for the proper interpretation of a disease process.

SUMMARY

1. Biopsy studies of immediate and delayed skin reactions to tobacco and saline control wheals were studied in 10 patients with thromboangiitis obliterans, one of migrating phlebitis, and one with acute coronary thrombosis.

2. Eosinophiles, varying in numbers, were found in the histologic sections of the immediate urticarial wheals produced by the tobacco allergen in 6 of 8 cases of thromboangiitis obliterans and in one case of migrating phlebitis.

3. The biopsies of 2 patients with thromboangiitis obliterans and one 37-year-old patient with coronary artery thrombosis, who developed delayed reactions, showed an acute dermatitis.

4. The exciting rôle of tobacco in the 12 cases studied was corroborated clinically by arrest in the progress of symptoms following cessation in the use of tobacco. In one patient (Case 9) giving a delayed eczematous type of local reaction, a generalized eczema and exacerbation of the symptoms of thromboangiitis obliterans were induced by repeated subcutaneous injections of tobacco extract.

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PERIARTERITIS NODOSA*

Case Report

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SINCE 1866, when Kussmaul and Maier[1] first accurately correlated the clinical and pathologic findings in periarteritis nodosa, well over 200 cases have been reported in the literature.[2] The diagnosis of this rather uncommon condition has rarely been made ante mortem. Most of the ante-mortem diagnoses were made through incidental biopsy studies in exhaustive diagnostic searches. In a smaller group of cases the diagnosis was made ante mortem on the basis of the clinical course and development. The diagnosis ante mortem without the presence of clinically demonstrable nodules has rarely been made. Middleton and McCarter[2] report a case in which the clinical course led to the diagnosis of periarteritis nodosa, and in the absence of the typical subcutaneous nodules a biopsy of the gastrocnemius muscle was made six days before death, without the finding of the characteristic lesion. However, this patient showed the lesion at autopsy in vessels of practically every organ. A report by Alan Bernstein[3] records a case of periarteritis nodosa diagnosed ante mortem without the presence of nodules confirmed by muscle biopsy. A report by Drs. Cohen, Kline, and Young[4] contains a case record of one patient in whom the diagnosis was made ante mortem in the absence of nodules confirmed by muscle biopsy, and of another patient who was seen six hours ante mortem in whom a diagnosis was made of periarteritis nodosa in the absence of nodules, and this diagnosis was confirmed at autopsy.

Gruber[5] first directed attention to allergic inflammation, with reversible and irreversible changes in periarteritis nodosa. Three cases of periarteritis nodosa were reported by Kline and Young[6] in which the histologic study of the lesions involving the arterial walls demonstrated changes of both the reversible and irreversible type, due apparently to allergic reactions in a highly sensitized body. Cohen, Kline, and Young[4] add a fourth case to this series, in which they conclude that periarteritis nodosa is a manifestation of clinical allergy.

The following case is being reported as an addition to those cases of periarteritis nodosa in which the diagnosis was made ante mortem in the

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absence of subcutaneous nodules and confirmed by muscle biopsy, and further as an addition to those cases in which allergy must be considered as a definite etiologic factor.

CASE REPORT

T. W., a 23-year-old married white female, was first seen on November 14, 1936, through the courtesy of Dr. H. S. Applebaum. Her chief complaint was asthma. Since the age of 20 she had had nonseasonal nasal congestion for which on August 1, 1936, the right inferior turbinate was trimmed. This operative procedure proved of no help. Four weeks later she developed hoarseness and cough, and on November 1, 1936, she had her first attack of wheezing, which was of forty-eight hours' duration. Further history obtained was to the effect that she had had urticaria on one occasion six years previously. She had had a dermatitis of the hands several years previously which subsided upon the discontinuance of the use of Lifebuoy soap. She had had the usual childhood illnesses and frequent winter colds. There was no history of food, drug disturbances, or idiosyncrasies. There were no animal contacts. Her last menstrual period was in August, 1936. Family history revealed the information that one sister had urticaria and that her father had nonseasonal vasomotor rhinitis.

Physical examination revealed an apparently healthy appearing adult female, weighing 142 pounds, who was having a moderate amount of cough and dyspnea. The skin was negative other than marked areolar pigmentation around the nipples.

BLOOD COUNTS

DATE	R.B.C.	HGB. PER CENT	W.B.C. PER CENT	POLYS PER CENT	LYMPHS PER CENT	EO-SINOS PER CENT	BASO PER CENT	MONO PER CENT
11/14/36	4.62	86	7,400	78	10	11	1	
12/21/36	5.08	92	18,700	81	10	7	1	1
4/ 9/37	4.2	90	16,600	68	14	8	2	8
6/ 3/37	4.56	78	22,800	73	10	10		7
7/ 8/37			28,600	56	23	16		5
7/16/37	3.61	60	32,000	15	12	76		1
7/26/37			37,250	21	6	70	1	2
7/29/37	3.74	55	40,000	14	9	73		4
8/ 4/37	3.58	55	41,500	19	9	64	1	2
8/ 9/37	3.58		20,000	45	12	38	2	3
8/13/37	3.28	50	18,500	59	20	12	2	7
8/16/37			15,000	53	17	24	1	5
8/19/37	3.58	52	20,800	54	6	38		2
8/21/37			27,500	50	10	33	2	5
8/27/37	3.40	50	24,000	65	9	24	2	
9/ 2/37	3.17	54	22,500	61	4	33		2
9/16/37	3.42	52	24,000	55	9	32	2	2
9/23/37			24,800	67	6	26	1	1

Chart I.—Blood counts in patient cited.

Both inferior and middle turbinates were red and edematous, and there was a moderate amount of mucopus in both nares. The teeth were normal and the tonsils were small. The chest was hyperresonant, and there were scattered fine and coarse râles throughout the lung fields. By percussion the heart was normal in size, the apex rate was 90, and there were no audible murmurs. The blood pressure was 110 systolic, 74 diastolic. The fundus of the uterus could be felt midway between the umbilicus and the symphysis pubis. Upon transillumination both antra were cloudy. Laboratory examination showed normal urine findings. Blood serology was negative. Red blood count was 4,390,000, hemoglobin 85 per cent (Sahli), white blood count

7,400. Differential smear showed polymorphonuclear neutrophiles 78 per cent, lymphocytes 10 per cent, eosinophiles 11 per cent, basophiles 1 per cent (Chart I).

The patient at this time was tested to the commoner inhalants and foods, with the following positive findings: A moderate reaction to house dust and feathers, and slight reactions to pyrethrum, tobacco, rice, corn, banana, pike, and tuna fish. The patient was advised to observe dust and feather-free precautions in her home, and was further advised to eliminate from her diet the foods which showed positive skin tests. Treatment with house dust extract was then instituted. She was given approximately six injections of a 1:100 dust extract, but because her asthma was becoming increasingly more severe she was referred to Mount Sinai Hospital on December 21, 1936. At this time the findings were similar to the examination in November, with the exception that there was considerably more purulent nasal discharge and more edema of both middle turbinates. X-ray examination showed dense diffuse cloudiness of both maxillary sinuses and diminished aeration of the right posterior ethmoid cells. X-ray of the lungs showed a small calcification in the right upper lobe, with larger regional hilal calcification; moderate increase in the fibrovascular markings in the mesial portions of both lower lobes; no bronchiectasis or evidence of foci of parenchymatous infiltration in the lung fields. She was treated symptomatically with heat to the sinus region and was given adrenalin for her severe asthma, and codeine, potassium iodide, and ephedrine for her cough. There was definite improvement in her symptoms. On December 29 she had a bilateral antral puncture. The culture from the sinus washings showed a scant growth of *Staphylococcus aureus*, with a few colonies of hemolytic streptococcus and pneumococci Type IV. Skin tests with vaccines of the aforementioned organisms showed no reactions. She was discharged from the hospital December 30, definitely improved of cough and asthma.

While at home, in addition to treatment with house dust extract, she was given periodic injections of an autogenous vaccine made from the antral washings. She had a comparatively free period from asthma until April 5, 1937, when she developed an acute upper respiratory infection, following which she had severe coughing spells and moderately severe asthma. Because of her pregnancy it was advisable to readmit her to the hospital. She remained there four days, during which time her cough and asthma were readily controlled with small and infrequent doses of adrenalin and codeine.

She next returned to the hospital on May 19, 1937, in labor. At this time she advised us that since her discharge from the hospital she had been entirely free of all asthma. On May 20 she was delivered of a normal male baby. Her postpartum course was afebrile, and she had no respiratory difficulty until May 28, when she began to have mild asthma which was readily controlled with small doses of adrenalin. She left the hospital on May 31 and after being home one day began to have very severe asthma, necessitating her return to the hospital. On June 3 she was admitted to the medical service at which time her temperature was 102° F. She had a very severe cough and was markedly cyanotic and dyspneic. She showed signs of an acute nasopharyngitis, with considerable mucopus in her nose and nasopharynx. The chest was full of moist and crepitant râles. The blood pressure was 120 systolic, 76 diastolic. There were no abnormal urinary findings. The white blood count on admission was 22,800, red blood count 4,360,000, hemoglobin 78 per cent. The polymorphonuclear neutrophiles were 73 per cent, eosinophiles 10 per cent, lymphocytes 10 per cent, myelocytes 7 per cent (Chart I). She was given avertin anesthesia of 60 mg. per kg. which gave her considerable relief from the severe asthma. She was also confined to an oxygen tent at intervals. X-ray examination of the sinuses at this time showed considerable cloudiness of both antra. However, bilateral antral puncture yielded no return other than a small particle of mucus obtained from the left antrum. This was further cultured and no growth

obtained. The very severe asthma gradually subsided. However, the patient had considerable cough and nasal congestion and ran a low grade febrile course of 99° to 100° F. Frequent sputum examinations failed to show anything other than a few pus cells and ordinary mouth organisms.

Physical examination on June 23 revealed impaired resonance over the right middle lobe, with increased voice and tactile fremitus; fine crepitant inspiratory râles were also heard over this area. Chest x-rays showed small patches of infiltration in the lateral inferior portion of the right middle lobe. The x-ray impression was patchy bronchopneumonia of the right middle lobe.

At this time her temperature started to show a daily afternoon level of 102° F., with a morning level of 100° to 101° F. She had persistent tachycardia, profuse night sweats, and began to complain of severe weakness, nausea, and loss of appetite. The pulmonary findings remained the same. Blood cultures were taken daily

Fig. 1.—Section from gastrocnemius muscle showing edema of muscle.

and were reported negative after several days' incubation. The sputum findings were the same as on the previous examinations. It was interesting to note that the patient no longer had asthma or dyspnea and that she had had her last injection of adrenalin on July 2.

On July 7 a firm nontender spleen was felt about two fingerbreadths below the left costal margin. Chest x-ray on July 8 reported several rounded dense areas in the right posterior axillary line. Fluoroscopic examination to localize these areas reported them as several nodules of infiltration, apparently in the right middle lung fields. The patient at this time began to complain of vague pains in her wrists and arms. The temperature was raising daily to 103° and 104° F. Blood cultures were still negative. On July 16 one was able to hear an accentuated first sound with a musical systolic murmur at the apex. At this time there was a marked change in the patient's blood picture. The red blood count had decreased to 3,610,000, the hemoglobin was 60 per cent, the white blood count had risen to 32,000,

and the differential smear showed 76 per cent eosinophiles, 15 per cent polymorphonuclear neutrophiles, and 12 per cent lymphocytes (Chart I).

The high temperature, the persistent high white blood counts with insufficient evidence of any localized infection, the unusually high eosinophile count, the development of a secondary anemia, the progressive weakness, and the vague muscle pains in a patient who was a known allergic with definite allergic symptoms suggested a diagnosis of periarteritis nodosa, and for that reason a muscle biopsy was done. The report of this biopsy by the Pathology Department, Mount Sinai Hospital, was as follows: "Slight edema of the muscle. Muscle fibers separated somewhat by edema and fine fibrous septae. For the most part the nuclei appear normal in number and staining quality. Occasional small areas show apparent absence of nuclei suggesting slight degenerative change. Vessels of average prominence, walls of average thickness, slightly engorged. Occasional vessel shows walls with media apparently hyalinized. No appreciable cellular infiltration. Final diagnosis: Slight edema of muscle" (Fig. 1).

Although this report of the biopsy failed to confirm the diagnosis of periarteritis nodosa, the thought was not abandoned. It was decided, therefore, to wait and observe the patient further, and do further muscle biopsies at a later date. Because of the high eosinophile count the stools were frequently examined for ova and parasites, but none were found.

The high fever, the exhaustion, the increasing anemia, the presence of an enlarged spleen, and the high white blood count with 76 per cent eosinophilia brought up the question of an acute eosinophilic myelosis. There were, however, no immature elements in the peripheral blood. A bone marrow examination through sternal puncture was done. This revealed an eosinophilic reaction with a marked increase of the eosinophiles, stabs, and mature neutrophiles, without any increase of the myeloblasts or myelocytes. All of this suggested a marked reaction to a severe infection in a highly allergic individual.

An interesting x-ray report of July 24 was as follows: Both lung fields show a diffuse seeding with fine nodules along the bronchial trees; no definite confluent foci of infiltration; the bases are more involved than the upper lobes. Impression: diffuse bronchiolitis. (Has this patient periarteritis nodosa?)

In addition to the findings already mentioned the patient developed a pustular rash on her face and on the extensor surface of her arms and legs. She had been taking sodium bromide, and this was diagnosed as bromoderma. The drug was discontinued and the rash promptly disappeared. Culture of the pus from these lesions showed a growth of *Staphylococcus aureus*. On July 30 there appeared several discrete purpuric lesions on the palms of her hands and soles of her feet. These lesions were macular, faded on pressure, and were associated with severe pruritus which was not relieved by either ephedrine or adrenalin. She also showed a few petechial hemorrhages in both conjunctivae and about the neck and thorax. The bleeding time and clotting time were within normal limits, and the blood platelet count was 511,000. The finding of these purpuric spots and petechial hemorrhages recalled another case of periarteritis nodosa which had been seen in this hospital and which was reported by Drs. Cohen, Kline, and Young.[4] It is of interest to note that at this time the pulmonary findings had practically entirely disappeared, with the exception of an occasional râle audible over the right middle lobe. By August 7 the purpuric lesions had started to fade, and at this time the patient had developed a gallop rhythm with a louder systolic murmur at the apex. An electrocardiogram showed a normal sinus rhythm, right axis deviation, inversion of T_1 and upright T_4. This was reported as indicative of myocardial damage.

On August 11 the patient showed moderate edema of her hands, feet, ankles, and sacral region, and some slight puffiness about the eyes. The liver was palpably en-

larged. Blood pressure at this time was systolic 150, diastolic 92. The urine was reported as showing two-plus albumin, specific gravity 1020, but otherwise normal. Blood chemistry revealed sugar 113 mg. per 100 c.c. blood, nonprotein nitrogen 32, urea 11.6, and creatinin 1.5. Because of the evidence of edema she was given salyrgan intravenously, with increased urinary output and some decrease in visible edema.

Her complaints at this time were weakness, nausea, loss of appetite, occasional diarrhea, vague pains in her chest, arms and legs, and profuse sweating. The blood picture at this time showed a decrease in the total white blood count to between 15,000 and 18,000. There was also diminution in eosinophiles to 12 per cent. The red blood count and hemoglobin remained approximately the same as on previous examinations (Chart I). X-ray report on August 21 showed considerable mottling throughout the right middle lobe, with thickening of the pleura between the middle

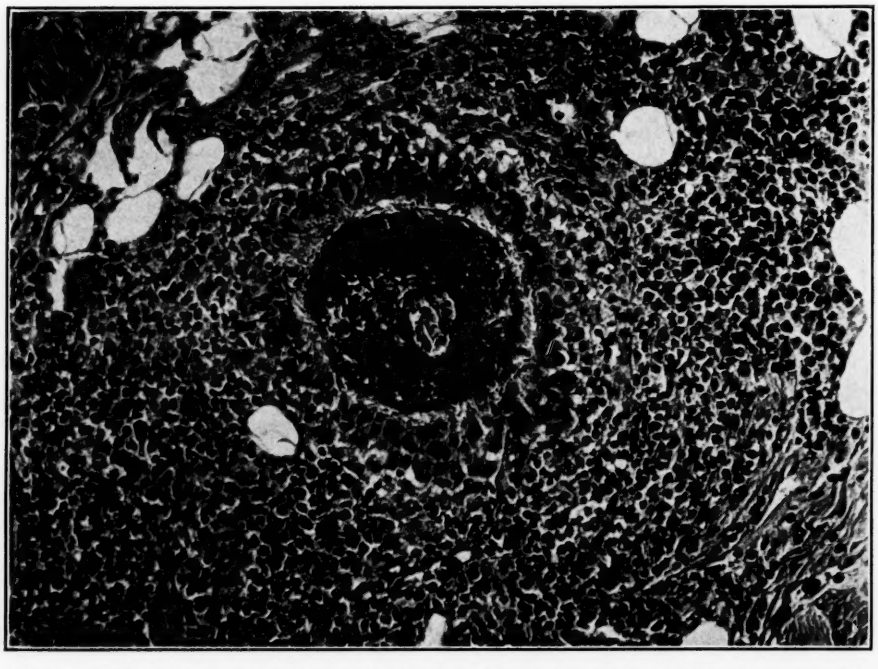

Fig. 2.—Section from pectoral muscle showing edema of muscle, thickened vessel wall, medial necrosis, numerous eosinophiles and round cells.

and lower lobes. The cardiac shadow was considerably enlarged and suggested the diagnosis of a possible pericardial effusion. Blood cultures which had been taken at repeated intervals were all negative.

On August 22 the patient left the hospital by release. While at home the edema became more marked, the urinary findings showed four-plus albumin with specific gravity of 1022, a large number of white blood cells and a moderate number of red blood cells and casts. The blood pressure rose to 180 systolic and 120 diastolic. The blood picture was approximately the same. The red blood count 3,400,000, hemoglobin 50 per cent, white blood count 24,000, but the eosinophiles were now 24 per cent (Chart I). The patient was referred back to the hospital on September 1 where she was given salyrgan and hypertonic sucrose with an excellent clinical response. The edema gradually disappeared and the liver decreased in size. By September 11 her blood pressure was 140 systolic and 90 diastolic. She was quite

comfortable and had no cough. She still continued to have fresh crops of petechial hemorrhages and purpuric spots on the hands and soles of her feet.

On September 13 a second muscle biopsy was done with the following report by the laboratory of Mount Sinai Hospital: "Muscle bundles separated somewhat by edema. The cytoplasm shows no appreciable abnormality. The vessels in one area present a striking picture. There are two vessels with considerably thickened walls; the lumina in part filled with a fibrin clot. The media of the wall is thickened and in part necrotic. At the periphery one vessel shows cellular infiltration. The tissue about the vessel is apparently proliferating. The cells show numerous eosinophiles and numerous large round cells, some suggesting plasma cells, others large mononuclear cells. The tissue about the vessels appears coherent. It is not encapsulated. There are a number of additional smaller vessels which show no degenerative changes or thickening of the media. Several areas of infiltration by wandering cells similar to those mentioned above are regional to the vessels. Final diagnosis: periarteritis nodosa" (Fig. 2).

The patient clinically was going downhill quite rapidly. She had lost considerable weight. Her temperature still varied from 101° to 104° F. She had increasing drowsiness, vague abdominal pains, and frequent stools. The blood picture of the patient did not change materially. The red blood count was 3,170,000, hemoglobin 54 per cent, white blood count 24,000, and eosinophiles varied from 26 per cent to 33 per cent. For the first time the blood chemistry showed increased nitrogenous retention. The nonprotein nitrogen was 65 mg. per 100 c.c. blood; urea 34.4; creatinin 2.5; uric acid 3.45. On September 29 the patient became quite stuporous. She had considerable hiccoughing, became semiconscious, and expired in uremia. An autopsy was not permitted.

DISCUSSION

The symptoms of this case are multiple and varied as in the other cases of periarteritis nodosa reported in the literature. Such protean symptoms should be expected, since the arterial supply of any organ may become affected by this disease process, the particular character of symptoms depending upon the vessels which happen to be involved.

Here the sequence of events and dominating symptoms induced were as follows: An extremely allergic individual with hypertrophic rhinitis, sinusitis, bronchitis, asthma, pulmonary emphysema who subsequently developed a bronchopneumonia which ran a very prolonged course. At this time the infection was the dominating feature. This was then followed by enlargement of the spleen with a high white blood count and marked eosinophilia. The next feature was myocardial damage with tachycardia, subsequent gallop rhythm, and systolic murmur with the electrocardiographic evidence of myocardial damage. This phase was then followed by a period when petechiae and purpuric hemorrhages, with incidental normal platelet count, were prominent signs. Abdominal symptoms with nausea, vomiting, diarrhea, and loss of appetite were also prominent in the course of the illness. Pains in the skeletal muscle were likewise present. However, the pains in the extremities were never as pronounced as in another case previously cited,[4] in which there was severe peripheral polyneuritis with subsequent foot-drop. In spite of the fact that the pulmonary findings finally cleared up the fever con-

tinued, ranging between 101° and 104° F. The height of the white blood count and the increased eosinophilia ran a course parallel with the fever and the size of the spleen. Later the kidneys became involved, with the development of edema, hypertension, nitrogenous retention, and the findings of albumin, red blood cells, white blood cells, and casts in the urine. The kidney damage became more and more severe with increasing uremia which finally terminated life.

Numerous theories of the etiology of periarteritis nodosa have been proposed among which are (a) syphilis; (b) virus (Harris and Friedrichs,[7] Arkin[8]) (c) rheumatic fever (Ophüls,[9] Friedberg and Gross,[10] Middleton and McCarter[2]); (d) allergy (Gruber,[5] Cohen, Kline, and Young[4, 6]). The latter authors conclude that periarteritis nodosa is a manifestation of severe clinical allergy to the extent that irreversible destructive lesions occur in the blood vessels and thus lead to the disturbances in the organs supplied by the involved vessels. The case here reported fits in very well in the latter etiologic classification.

SUMMARY

A case of periarteritis nodosa is reported in an allergic individual in whom it is believed that allergy was of etiologic importance. The diagnosis was made ante mortem and in the absence of clinically demonstrable subcutaneous nodules and was verified by muscle biopsy.

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THE EFFECTIVENESS OF INTRAVENOUS HYPERTONIC SUCROSE AND ADRENALIN IN THE TREATMENT OF STATUS ASTHMATICUS*

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THE patient in status asthmaticus is an acute medical emergency for which there is no satisfactory method of treatment. A measure which will relieve the grave respiratory distress immediately, even though temporarily, may be lifesaving.

The bronchi and bronchioles, in bronchial asthma, are partially obstructed by exudate, by muscular contraction, and by swelling of the mucosa. This partial obstruction must be more marked in status asthmaticus. The difference in degree of obstruction is probably due to an increase in the exudate and in the swelling of the mucosa. Naturally, such reasoning is purely theoretical. Therapy, nevertheless, should be directed toward the alleviation of the obstruction.

It is necessary for the patient to relieve himself of the exudate by coughing. Adrenalin, because of its constrictive effect on the blood vessels and its dilating effect on the smooth musculature of the bronchial tree, gives relief in the ordinary attack of asthma. Adrenalin is often inefficient in status asthmaticus and the patient, therefore, is generally considered to be adrenalin-fast. However, it is more logical to believe that the partial bronchial and bronchiolar obstruction has developed to such a degree that adrenalin alone is insufficient. The mucosal swelling should be lessened by increasing the osmotic pressure in the circulating blood with some hypertonic solution, thereby drawing the excess fluid from these tissues into the blood stream.

The effects of various hypertonic solutions on the cerebrospinal fluid pressure have been extensively studied. The observations on animals by Bullock, Gregersen, and Kinney[1] and on human beings by Masserman[2] and Hahn and his associates,[3] indicate that the secondary rise in the cerebrospinal fluid pressure following the use of hypertonic glucose and saline solutions is avoided by using hypertonic sucrose solution. The osmotic effect of such substances as sodium chloride and glucose, which pass through the capillary wall with ease, is curtailed by diffusion. Sucrose remains in the blood a longer time after injection because its molecules are larger and less diffusible. Furthermore, it is not broken down to any significant extent or utilized as glucose, nor stored in the tissues as is sodium chloride (Bullock, Gregersen, and Kinney[1]).

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Masserman[2] studied the effects of the intravenous administration of 50 per cent sucrose solution in 35 patients and, according to his results, its injection at the rate of 5 to 10 c.c. a minute, in amounts no more than 500 c.c. is a safe procedure.

The following are brief case reports of six patients who were admitted to the hospital in typical status asthmaticus. Each patient received one or more intravenous injections of 100 c.c. of 50 per cent sucrose to which was added 0.5 c.c. of 1:1000 adrenalin hydrochloride. In this small group of patients, the combination of sucrose and adrenalin seemed more efficacious than sucrose alone or adrenalin given intravenously with hypertonic glucose. In the remainder of the article, unless otherwise specified, the expression sucrose and adrenalin is used to imply a mixture of 100 c.c. of 50 per cent sucrose and 0.5 c.c. of 1:1000 adrenalin hydrochloride.*

CASE 1.—R. L., a 44-year-old married man, was known to have suffered with asthma sporadically for the past twenty years. For three weeks prior to his admission to the Johns Hopkins Hospital, he had asthma which was relieved only momentarily by adrenalin. For two days prior to his admission, adrenalin had failed to give relief. Following admission, he was given sucrose and adrenalin intravenously; and before the injection was completed, his asthma had subsided. There was gradual reappearance of his former symptoms in the next fifteen minutes; but thereafter, the asthma gradually disappeared, and in thirty to sixty minutes he was entirely asymptomatic. The examination of the chest at this time still revealed the presence of musical râles, but it was the impression of four observers that there had been a definite decrease in the physical findings. Three to four hours later, he complained of "tightness in the chest." Relief was afforded by 0.2 c.c. of adrenalin given every fifteen minutes for three doses. He remained comfortable for six hours, when it became necessary to repeat the former course of adrenalin. During the remainder of his five-day period of hospitalization, he was entirely free from asthma.

CASE 2.—D. D., a 20-year-old white single girl, was known to have had asthma "all her life." For six days prior to her admission to the Johns Hopkins Hospital, she had asthma which was alleviated temporarily by adrenalin; but for two days before her admission, adrenalin had been ineffective. Immediately on admission she was given sucrose and adrenalin intravenously. Following the injection, she complained of a "feeling of numbness all over" and developed a typical carpopedal spasm which disappeared spontaneously in five to ten minutes. The asthma disappeared concomitantly with the carpopedal spasm, and on examination of the chest the lungs were found to be entirely clear. This observation was confirmed by two different observers. Six hours later she complained of a "mild tightness in the chest," which was completely relieved by 0.2 c.c. of adrenalin. During the next

*In administering the sucrose and adrenalin, I have used a 20 c.c. syringe to which is attached a two-way stopcock. A 20-gauge needle is attached to the vertical arm of the stopcock and a short piece of rubber tubing, which has previously been treated with sodium bicarbonate and acetic acid, is attached to the horizontal arm of the stopcock. It is through this tubing that the solution is withdrawn from its container into the syringe. Thus, during the course of the intravenous administration of the solution, the syringe can easily be refilled, as necessary, without withdrawing the needle from the vein.

Because the 50 per cent sucrose is very viscous, the solution should not be given by the gravity method.

two days, she had two mild attacks of asthma, each of which was relieved by 0.3 c.c. of adrenalin. Thereafter, during her stay in the hospital, she was free from asthma.

Six weeks later the patient was seen in the office of Dr. Leslie N. Gay in status asthmaticus. Intravenous sucrose and adrenalin gave relief immediately, and she left the office three hours later asymptomatic. She was not heard from for six days, when she returned to the office again in status asthmaticus. On this occasion, unknown to the patient, she was given 100 c.c. of 50 per cent glucose to which was added 0.5 c.c. of 1:1000 adrenalin hydrochloride. She experienced the same temporary amelioration that she had following the two previous injections of sucrose and adrenalin, but in one hour severe asthma reappeared, and she stated herself that the injection had not helped her as it had done previously. She was readmitted to the hospital and given sucrose and adrenalin two hours later; but the solution was administered too rapidly. Immediately, the patient complained of a severe shooting pain behind the eyes; and, on examination, the eyeballs were found to be abnormally soft. This pain persisted throughout the night, although the asthma had ceased. From then on, the asthma was adequately controlled by single doses of adrenalin, and she was discharged six days later, free from symptoms.

CASE 3.—E. W., a 27-year-old single white woman, was admitted to the Johns Hopkins Hospital in typical status asthmaticus. She was given 100 c.c. of 50 per cent sucrose from which there was only slight relief. Two hours later, she was given sucrose and adrenalin from which there was immediate and complete relief of symptoms for three hours. In the following twelve hours, 0.5 c.c. of adrenalin was given three times, but during the next day, only one administration was necessary. Thereafter, and until her discharge four days later, she remained asymptomatic.

CASE 4.—M. C., a 29-year-old colored married man, was known to have had asthma for the past nineteen years. The present attack began six days prior to his admission to the Johns Hopkins Hospital. For twelve hours before his admission, adrenalin had failed to give relief. He was given sucrose and adrenalin intravenously, and there was immediate relief of symptoms for the following two and one-half hours, when asthma gradually reappeared. He was given 2.5 c.c. of adrenalin in divided doses without relief and then, 0.1 c.c. of adrenalin every thirty seconds, until 1.0 c.c. had been given. Although he was better, the asthma persisted. The following day, he was found in such a severe attack that the interne wrote, "Each gasp seemed as if it would be his last." He was given sucrose and adrenalin with immediate and lasting relief.

CASE 5.—M. M., a 24-year-old white married woman, was admitted to the Johns Hopkins Hospital in status asthmaticus which had been present for the past two days. She was given 1.0 gm. of calcium gluconate intravenously and then, 50 c.c. of 50 per cent glucose, without effect. She felt better after being placed in an oxygen tent, but the asthma continued. At the end of twelve hours, during which time her condition remained unchanged, she was taken from the oxygen tent and given sucrose and adrenalin intravenously. There was immediate relief, and thirty minutes after the injection she slept for the first time in three days. In three hours the asthma reappeared and the sucrose and adrenalin administration was repeated. Relief followed the injection and she remained comfortable for seven hours, when it became necessary to repeat again the sucrose and adrenalin. After 70 c.c. of the mixture was given, she complained of a feeling of nervousness and the injection was discontinued. Mild asthma persisted for the following seven hours and then disappeared entirely. During the following two days of hospitalization, she remained asymptomatic.

CASE 6.—J. D., a 30-year-old white married man, was known to have had asthma "for years." The present attack began four days prior to his admission to the Johns Hopkins Hospital and had persisted despite numerous injections of adrenalin and the frequent use of an adrenalin nebulizer. He was given 2.0 gm. of sodium iodide intravenously, and in thirty minutes, he stated that he felt worse. Sucrose and adrenalin were given with only fleeting relief. After being placed in an oxygen tent, he became more comfortable, but the asthma continued. After twenty-four hours, he was taken from the oxygen tent and treated with frequent injections of adrenalin. Twenty-four hours later, the adrenalin failed to relieve the attacks and he became definitely worse. He was put back into the oxygen tent, without any change in his condition. Sucrose and adrenalin were given, and immediately the asthma disappeared. Concomitantly, there was a complete evanescence of all the physical signs of asthma. He continued to remain comfortable, with the exception of an occasional mild attack which was relieved by adrenalin.

SUMMARY AND DISCUSSION

Six patients in status asthmaticus were treated intravenously with hypertonic sucrose solution and adrenalin. Each patient received one or more injections of 100 c.c. of 50 per cent sucrose to which was added 0.5 c.c. of 1-1000 adrenalin hydrochloride. Twelve injections were given to the six patients and, with only two exceptions (in one instance, when the administration was discontinued because the patient complained of nervousness; and in the other, when the injection followed the intravenous administration of sodium iodide) there was immediate relief from asthma which persisted at least from two and one-half to three hours.

One patient (Case 6) was given 2.0 gm. of sodium iodide intravenously thirty minutes before the sucrose and adrenalin. Theoretically, sodium iodide should increase the bronchial and bronchiolar obstruction by increasing the amount of exudate. Following the administration of the sodium iodide, the patient did seem worse and the fleeting relief that followed the injection of sucrose and adrenalin was attributed to the adrenalin. Two days later, during a similarly severe attack of asthma, the sucrose and adrenalin gave striking relief which persisted for three hours.

Fifty per cent glucose was substituted for the 50 per cent sucrose in one patient (Case 2) who had received on two previous occasions definite relief from the sucrose and adrenalin. Severe asthma reappeared after one hour of relief. Such an adverse effect might be anticipated because glucose is broken down and utilized by the body and because its osmotic effect is mitigated by its ability to diffuse through the capillary walls. Glucose, having diffused through the capillary walls, withdraws fluid from the blood stream into the surrounding tissues, and the end result is an increase instead of a decrease, in the mucosal swelling. The same reasoning is applicable to solutions of sodium chloride.

Sucrose without adrenalin was given to one patient (Case 3). There was a little relief, but not the dramatic effect that followed the admin-

istration of sucrose and adrenalin three hours later. Because adrenalin is rapidly removed from the blood stream by the liver,[4] it is difficult to believe that the adrenalin alone is responsible for the amelioration of symptoms which last for three hours or more. The dangers of giving adrenalin intravenously are practically abolished by its dilution in the sucrose and by the cautious administration of the solution.

Two patients (Cases 1 and 2), during their first period of hospitalization, received but one injection of sucrose and adrenalin. Each developed mild attacks of asthma, in twelve to forty-eight hours following the injection, which were controlled by small doses of adrenalin.

Two patients (Cases 4 and 6) received two injections at intervals of twenty-four and seventy-two hours, respectively. In the former, the second injection of sucrose and adrenalin heralded the abatement of symptoms and thereafter, the patient remained asymptomatic. In the latter, asthma reappeared sporadically, but was adequately relieved by single doses of adrenalin.

One patient (Case 5) received three injections of sucrose and adrenalin at three- to six-hour intervals. The asthma slowly abated following the last injection, and twelve hours thereafter, the patient was entirely asymptomatic.

One patient (Case 3) received sucrose and adrenalin three hours after she had been given sucrose alone. In the twelve hours following the last injection, she received three doses of adrenalin, and in the next twelve hours, only one. Thereafter, she remained asymptomatic.

Prior to the administration of sucrose and adrenalin, each patient was considered to be adrenalin-fast. The attacks of asthma that appeared after the patient had received sucrose and adrenalin were adequately controlled by single doses of adrenalin. It is reasonable to believe, therefore, that the patients in status asthmaticus are not adrenalin-fast, but that the adrenalin alone is incapable of relieving the degree of bronchial and bronchiolar obstruction that exists.

Five patients (Cases 1, 2, 3, 4, and 5) were entirely free from asthmatic symptoms in twenty-four to thirty-six hours after the first injection of sucrose and adrenalin, and they continued asymptomatic during the remainder of their period of hospitalization. One patient (Case 6) continued to have mild attacks of asthma.

Untoward symptoms referable to the treatment developed in two patients. Following the administration of sucrose and adrenalin, one patient (Case 2) developed a carpopedal spasm that disappeared spontaneously in five to ten minutes. The same patient, on another occasion, complained of a sharp pain behind her eyes and, on examination, the eyeballs were found to be abnormally soft. However, on both occasions, the injection was given too rapidly. The other patient (Case 5) complained of nausea and a feeling of nervousness after 70 c.c. of the third

injection had been given. Although these reactions emphasize the care with which such a solution must be given, they do not contraindicate its usage.

It is my intention to continue this study by substituting saline and glucose solutions for the sucrose. Larger amounts of sucrose will be given and the results compared with those obtained from this group of patients that I have just reported. Although the results have been gratifying in the six patients already treated, further study is necessary to support adequately the theory upon which such therapy is based.

I wish to express my gratitude to Dr. Leslie N. Gay, whose interest has made this study possible.

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INSULIN HYPERSENSITIVITY*

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A NUMBER of excellent observations of the interesting phenomenon, insulin hypersensitivity, have appeared in the medical literature. Most authors have considered such hypersensitivity due either (1) to the insulin protein itself, (2) to the animal protein from which the insulin was obtained, (3) to some extraneous substance of the pancreas not removed in manufacture, and since the advent of the newer protamine zinc insulin, (4) to the chemical or protein reaction to these added substances.

Again, the numerous reports of a severe local or general reaction following the initial injection of insulin would suggest the possibility of an inherited allergy or atopy, whereas another group of case reports and certain animal experiments have demonstrated the sensitizing power of insulin protein and the possibility of a true anaphylaxis following its repeated administration.

Recently we have seen two cases of so-called insulin hypersensitivity, the first representative of a hypersensitivity to the animal protein from which the insulin was obtained and the second illustrative of a hypersensitivity to the extraneous protein contained in the commercial insulin.

CASE REPORTS

CASE 1.—V. S., a tubercular white female, aged twenty-seven years, was seen by courtesy of Dr. Louis Baer. This patient had been given injections of Lilly's U 20 pork insulin so that she might gain weight. The first and each succeeding injection of 10 unit doses caused hives and irritation at the point of injection. After 12 injections, the treatment had to be discontinued. This patient had had no previous allergic manifestations and there was no family history of allergy.

The patient was tested by scratch and intradermal methods to 150 of the usual allergenic test materials. The only positive test was that to pork. Scratch and intradermal tests to various commercial brands of pork insulin showed positive reactions, but tests to the various beef insulins and to crystalline insulin were negative.

The patient was desensitized to pork insulin by starting with one unit and increasing the daily injection by one unit until 15 units could be taken without local reaction.

CASE 2.—H. G., a white female diabetic, aged forty-four years, showed marked local swelling and hives following the first and each succeeding injection of Lilly's U 20 pork insulin. The tenth injection caused intense urticaria and hives particularly at all the sites where the insulin had been injected previously. The patient had had no previous manifestation of allergic symptoms nor was there a family history of allergy.

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Scratch and intradermal tests to 150 of the usual allergens were entirely negative. The patient showed marked positive test, however, to various commercial beef and pork insulins even when diluted to the extent that 1 c.c. represented one unit of insulin. Tests made with Lilly's U 40 protamine zinc insulin, with a special Lilly's U 40 beef protamine zinc insulin, and with Parke-Davis deinsulinized pancreas were found strongly positive. A positive passive transfer test to Lilly's U 20 insulin was found in one of the two normal individuals injected with the patient's blood serum. Tests to human pancreatic extract, protamine zinc buffer, crystalline insulin, pituitary extract, thyroid extract, and ovarian extract were negative.

Since tests with crystalline insulin were negative but tests to pork pancreatic extract were positive, tests were made with two other enzymes of the pancreas; namely, trypsin and amylopsin. The test materials used were "Injectio Trypsini" and "Injectio Amylopsini" furnished by courtesy of Fairchild and Co., the former a solution containing trypsin separated from the associated enzymes of the pancreatic gland so as to have only a trace of amylopsin (pancreatic diastase) and practically free from lipase; and the latter the pancreatic diastase practically free from all other pancreatic enzymes. These materials were diluted so as to be isotonic. Scratch tests to the trypsin extract were negative, and intradermal tests gave questionably positive tests. Scratch test to the amylopsin extract showed a 30 mm. reaction and 0.02 c.c. of the solution injected intradermally resulted in a marked local reaction extending from the shoulder to the elbow and persisting for several days. Control tests with the trypsin and amylopsin were negative.

Attempts were made unsuccessfully to desensitize the patient to commercial insulin, but she was able to take the crystalline insulin without difficulty.

A very comprehensive review of Insulin Allergy was reported in the JOURNAL OF ALLERGY in 1934 by Marion T. Davidson.[1] He concluded, (1) The purest insulin obtainable at present shows definite allergenic and antigenic qualities and hence is protein in character. (2) Insulin from animal sources is different from the human variety as it is inconceivable that man would become sensitive to a product of his own body. (3) Sensitization to insulin is comparatively rare. (4) The rarity of this sensitization together with the rarity of the coexistence of diabetes and allergy is sufficiently pronounced to suggest the possibility of a causal relationship.

Since the work of one of us (F. S.[2]) in which sensitivity to human pituitrin was found, it is conceivable that man could be sensitive to human pancreas. We did not find this true, however, in the two cases which are reported here nor have we knowledge of any reported cases. The fourth conclusion, namely, the coexistence of diabetes and allergy being sufficiently pronounced to suggest the possibility of a causal relationship is hardly tenable since the publication of more recent work.[3]

In the September, 1934, JOURNAL OF ALLERGY, Murphy and his coworkers[5] reported two cases of insulin hypersensitivity, both occurring about seven days after the primary dose of insulin. In one the major symptom was urticaria and the other was asthma. One patient was a known allergic and the other was not.

Tuft[4] in 1928 reported two cases of insulin hypersensitivity, one he concluded was caused by the insulin protein itself and the other by some extraneous protein in the commercial insulin.

Regarding the antigenic properties of insulin, Lewis[5] stated: "The greater prevalence of hypersensitivity to insulin preparations when they first began to be generally used as compared to substances now in use is probably to be attributed to the presence of extraneous substances that have for the most part been eliminated with the development of better processes of manufacture. Nevertheless reactions, both general and local, do still occur with the refined substances and present a baffling problem in the treatment of diabetes.

Commercial preparations of insulin are derived from either beef or pork tissues or indiscriminately from both. Studies of cases of hypersensitivity to insulin show that most of them are sensitive to the insulin protein itself, although some show sensitization to the protein of the species from which the insulin was prepared."

In testing for anaphylactic sensitization with the Schultz-Dale Technique, this author used beef and pork insulin, stating, "While neither was actually crystalline material they were 'practically' as pure as crystalline insulin."

While it is true that the nitrogen content of the crystalline preparation of insulin and that of the commercial product are practically the same and that the commercial companies do feel that their regular commercial insulin is "almost" as pure as crystalline insulin, nevertheless since there is undoubtedly some extraneous material present in commercial insulin there is a question whether or not Lewis in his experiment has shown an actual insulin hypersensitivity or an anaphylaxsis to some extraneous protein in the commercial insulin preparation used.

In the *Journal A. M. A.* August 28, 1937, J. S. Dugan reported a case of a diabetic woman who received from 6 to 8 injections of insulin of from 20 to 30 units twice daily. He stated, "After each injection there was localized redness, slightly indurated, about the size of a silver dollar (38 mm.) which itched intensely. The final injection caused a general reaction, which was relieved only after the injection of 1 c.c. of epinephrine." He asked, "what probably caused this reaction?" The editor replied as follows: "The patient is probably allergic to the traces of protein in the insulin that is being used. This insulin, as prepared for general distribution, is made from hog pancreas. The same firm can supply insulin derived from beef pancreas, which the patient will probably be able to take without trouble."

The objection to this answer is that a patient sensitive to some extraneous protein in the insulin preparation would probably be sensitive regardless of the origin of the insulin. Again in assuming that the patient is sensitive to the traces of protein in the insulin rather than

to the insulin itself disagrees with the opinion of Lewis[5] who stated "Studies of cases of hypersensitivity to insulin show that most of them are sensitive to the insulin protein itself, although some show sensitization to the protein of the species from which the insulin was prepared."

Bryce[6] reported a case showing an extreme degree of hypersensitiveness to insulin and found that similar reactions were given by insulin from pig, ox, and sheep pancreas as well as several brands of commercial insulin. The author considered it highly probable that in his case the insulin itself and not some contaminated substance was the active agent in producing the allergic reaction. He apparently did not test the patient with crystalline insulin.

Berger and Hansen[7] stated: "The allergic reactions occur always after protracted use of insulin, never after a single injection. This points to the fact that a congenital idiosyncrasy in the old clinical sense is not involved in any case, but always a true acquired allergy. The symptoms correspond to those which are observed in cases of allergic diathesis."

From their experiences they drew the tentative conclusion that the antigenic substance in commercial insulin is evidently very stable, is an albuminose or peptone, or perhaps belongs to the group of specific carbohydrates. They stated: "It appears that the conclusion of Campbell regarding the hypersensitiveness to insulin, as such, is not justified, since even these crystalline insulins cannot be regarded as chemically pure."

Karr, Scull, and Petty[8] reported a case in which the patient was sensitive from the very start of injections. While she was originally sensitive to crystalline insulin, after a period of about one year in which no further insulin therapy was given, she was again tested and found sensitive only to regular insulin and not to crystalline insulin. They stated: "Why she was originally sensitive to crystalline insulin and now only to commercial insulin is an interesting speculation."

Kraeupl[9] stated: "Authors disagree concerning the nature of the antigen contained in insulin, but the majority believe it to be a protein mixture or a substance belonging to the group of specific carbohydrates; some accuse the conserving medium. The sensitizing power of insulin was proved in the animal experiment."

He further remarked: "The fact that insulin allergy is very rarely observed, as compared with the frequency of insulin medication in courses at long intervals, that is, under conditions favoring the creation of hypersensitivity, suggests for its appearance the cooperation of an individual factor which attenuates or delays the desensitizing effect of single injections in rapid succession."

In a personal letter from Dr. F. B. Peck of Eli Lilly and Company he wrote as follows: "The problem of allergy to insulin is rather complicated, and we find certain individuals who are sensitive, not to insulins

derived from any particular protein source, but to insulin itself, even of the purest crystalline varieties. Fortunately, most of these patients encountered are fairly mild, and the majority of them seem to desensitize themselves spontaneously with the use of insulin over a period of several weeks. In those who react severely, one must occasionally follow such a procedure as was outlined by Bayer in the J. A. M. A. 102: 1934, 1934. We have encountered only a few cases which developed general manifestations of allergy to protamine zinc insulin. In these instances it is probably simpler to return the patient to some type of unmodified insulin. In a larger group, however, local sensitivity develops which is usually indicated by induration, swelling, and occasionally a little tenderness or erythema about the site of injection. Most of these individuals, however, desensitize themselves with no particular difficulty by simply continuing the injections over a period of a few weeks. If the local reaction is severe, it seems desirable to reduce the dose to a low level and control the diabetes in the meanwhile with unmodified insulin, gradually increasing the dose of protamine zinc insulin as desensitization occurs."

Again he stated in another letter: "Fortunately, most of the patients who are sensitive are fairly mild diabetics and although some discomfort may be caused for a period of a few weeks, it is usually possible to obtain sufficient hyposensitization to allow them to continue with their insulin injections."

In a third letter he discussed our Case 2 stating: "Since she (H. G.) does not react to the crystalline preparation, but does to the others, it would seem that some other factor must be responsible." Our experience with these cases, however, is that they usually, after a period of time, become less sensitive, even when using the original material. It is of considerable interest that the nitrogen content of the crystalline preparation and the other products is practically the same; in fact, we feel that the latter are about as pure as is the crystalline material.

During the past year and a half of investigation of protamine zinc insulin we have encountered a similar situation in that certain individuals develop local reactions due to the protamine content of the preparation. With this substance also, however, most of the patients apparently desensitize themselves within a period of several weeks and are then able to continue with their injections without any particular difficulty. Some of the investigators having large groups of patients under their observation have informed us that as a rule they simply disregard these phenomena, as they pass off in a few weeks.

DISCUSSION

A review of any number of case reports of insulin hypersensitivity must impress one with the considerable difference of opinion as to the underlying cause of this phenomenon. It is possible that each author

in his study of a necessarily limited number of cases has made deductions which are not applicable to a general consideration of the subject. Certainly it would seem that hypersensitivity might result from any one of the substances contained in the insulin material, whether or not it be the insulin protein itself, the animal protein from which the material is obtained, the extraneous products not removed in manufacture, or to such added substances as the protamine zinc buffer.

Again sufficient reports are presented to suggest that some individuals have a true inherited idiosyncrasy or atopy to insulin protein or to some extraneous material found in the insulin preparation, while other individuals may become sensitized after repeated injections.

The authors wish to express their appreciation to Dr. F. B. Peck, and to Eli Lilly and Co., both for their helpful suggestions and the liberal supply of materials.

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THE CLINICAL USE OF PHENYL-PROPANOL-AMINE HYDROCHLORIDE (PROPADRINE) IN THE TREATMENT OF ALLERGIC CONDITIONS*

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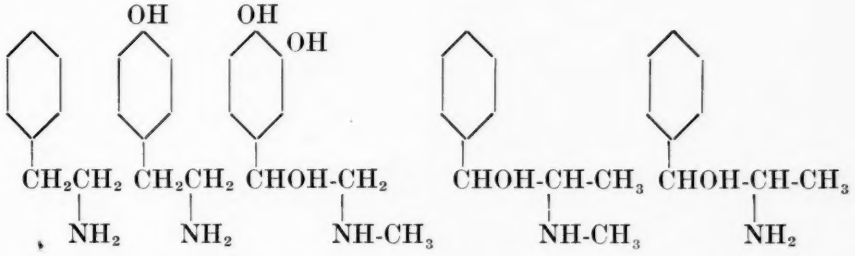
PHENYL-1-amino-2-propanol-1 is a member of a series of compounds structurally derived from B-phenyl-ethyl-amine and related to such valuable and active products, as tyramine, epinephrine, ephedrine, hordenine, etc.[1]



B-phenyl ethyl amine | tyramine | epinephrine | ephedrine | propadrine

This synthetic substance derived from propane is called "propadrine," the *propa* signifying the 3-carbon chain, and the *drine* showing its relationship to the ephedrine type of alkaloids. This designation is less confusing than the term "nor-ephedrine" and more convenient than "phenyl-propanol-amine."

The compound has been known for some time. Optically active forms have been found in small amounts in nature; l-propadrine[2, 3] and d-4-propadrine in ephedra species[4] and in *Catha edulis*.[5]

Three references to the clinical use of propadrine appear in the literature. Chen[6] administered it orally in 50 mg. doses to two individuals and found it to produce a maximum rise of 16 mm. and 30 mm. of mercury in the systolic blood pressure. Stockton, Pace, and Tainter[7] observed that topical applications of the solution to the nasal mucosa of patients with acute or chronic sinusitis showed no evidence of local irritation, whereas when ephedrine was similarly administered, 25 per cent of the patients complained of burning and irritation.

*From the Allergic Clinic, Episcopal Hospital, Philadelphia.

TABLE I

PATIENT NO.	ALLERGIC MANIFESTATIONS	DOSE OF PROPADRINE HYDROCHLORIDE $\frac{3}{8}$ GR. CAPSULES	RESULTS
1	Asthma	Two t.i.d.	Not quite so effective as other drugs, but did not cause urinary retention
2	Asthma	One or two p.r.n.	Much relief with 1 or 2 capsules
3	Asthma	Two t.i.d.	Marked relief; loosened mucus in chest
4	Asthma	Two b.i.d.	Same relief as with ephedrine
5	Pollen asthma	Two t.i.d.	Some relief; made sweat; not as nervous
6	Pollen asthma	Two p.r.n.	Some relief; made sweat; took 15 min. to work
7	Pollen asthma	One p.r.n.	Not nervous; much relief
8	Pollen asthma	Two t.i.d.	Relief. No bad attacks
9	Asthma	Two t.i.d.	Better than ephedrine. Made expectoration easier
10	Asthma and eczema	One q. 3 h.	Better than other prescriptions
11	Asthma	Two p.r.n.	Excellent relief, no insomnia
12	Pollen asthma	One q. 3 h.	Some relief
13	Pollen asthma	Two b.i.d.	Gave very marked relief with 1 capsule
14	Asthma	Two p.r.n.	Some relief, but no nervousness as from ephedrine
15	Pollen asthma	One t.i.d.	No relief
16	Asthma	One b.i.d.	Helped, especially the cough
17	Asthma	Two q.p.m.	Helped
18	Asthma	Two q. 3 h.	No relief
19	Asthma	Two p.r.n.	Fair relief
20	Pollen asthma	Two p.r.n.	Relief, and did not cause pain and difficulty of urination
21	Pollen asthma	Two p.r.n.	Helped, and no nervousness
22	Pollen asthma	Two p.r.n.	Helped, and no nervousness
23	Asthma	Two q. 3 h.	Relieved some
24	Asthma	One t.i.d.	Gave same relief as others
25	Asthma	Two p.r.n.	Very good relief. No nervousness
26	Asthma	One t.i.d.	More effectual than other measures used
27	Pollen asthma	Two p.r.n.	Gave fine relief

TABLE I—CONT'D

PATIENT NO.	ALLERGIC MANIFESTATIONS	DOSE OF PROPADRINE HYDROCHLORIDE ⅜ GR. CAPSULES	RESULTS
28	Pollen asthma	Two b.i.d.	Gave relief. No walking or talking in sleep, as with ephedrine
29	Asthma	Two q. 2 h.	Cleared chest and no difficulty on urination as with ephedrine
30	Asthma	Two q. 3 h.	Gave relief
31	Pollen asthma	One b.i.d.	Good relief
32	Pollen asthma	One p.r.n.	Relieved attacks and no insomnia
33	Pollen asthma	Two p.r.n.	Better relief than ephedrine and no nervousness
34	Pollen asthma	One t.i.d.	Much relief
35	Pollen asthma	One t.i.d.	Stopped wheezing and cough
36	Pollen asthma	Two p.r.n.	Relieved and no nervousness
37	Urticaria	One q. 3 h.	Better and no nervousness
38	Pollen asthma	One t.i.d.	Gave marked relief
39	Pollen asthma	Two t.i.d.	Same amount of relief. No retention of urine
40	Pollen asthma	One t.i.d.	Relief. No nervousness or insomnia
41	Pollen asthma	Two p.r.n.	Relief. No insomnia or nervousness
42	Asthma	Two p.r.n.	Marked relief
43	Urticaria	One t.i.d.	Relief, no palpitation, no tachycardia
44	Pollen asthma	Two q. 3 h.	Helped a great deal

Black[8] has reported the use of propadrine hydrochloride in the symptomatic control of hay fever and asthma in a series of 131 patients. In his clinical evaluation he found propadrine hydrochloride equally as effective as ephedrine salts, but it was relatively free from the side effects of insomnia, nervousness, motor restlessness, and nausea, so commonly experienced following the administration of ephedrine. Black also states, "The use of propadrine every three or four hours gave more relief to the patients suffering with urticaria and angio-neurotic edema than any other medication we have found."

The propadrine used in the present studies was the hydrochloride of the racemic dl-form, m. p. 190-1° C. The salt is readily soluble in water, and aqueous solutions appear to be stable over long periods of time.[9]

For simplicity, the 44 patients who cooperated in this clinical study are reviewed below in tabular form. In this study propadrine hydro-

chloride was used freely, and as much as ¾ gr. every two hours for five days or more was administered without any toxic manifestations. Previously, the patients had been taking ephedrine or other drugs for the control of their symptoms. All other drugs were withheld during the use of the propadrine hydrochloride. The patients were asked to comment on the relief obtained from propadrine hydrochloride as compared to any of the previous medicaments. Their replies appear in Table I under Results. Approximately one-half the patients were seen at Episcopal Clinic and the other half in private practice.

DISCUSSION

This table is self-explanatory, but in studying the patient's comments they may be divided into three groups:

1. Propadrine hydrochloride inferior to other forms of medication (usually ephedrine) in 4 cases (9 per cent)
2. Propadrine hydrochloride equally efficacious to previous medication in 5 cases (11 per cent)
3. Patients reporting greatest symptomatic improvement with propadrine hydrochloride, 35 cases (80 per cent)

Clinically, bronchial spasm, rhinitis, and sneezing were relieved, but in my experience the outstanding value of propadrine hydrochloride is the absence of side reactions, so commonly encountered in the administration of ephedrine. In a large number of cases the patients derived marked relief without the usual manifestations of nervousness and insomnia, a frequent complaint of patients when using ephedrine. It has not been found necessary, therefore, to administer sedatives with propadrine hydrochloride, as is frequently essential when ephedrine is employed.

It is well known that in the early use of ephedrine cases of hypertrophy of the prostate gland came to operation because of retention of urine, which we now know is often caused by ephedrine, since following withdrawal of ephedrine, the retention is relieved. This clinical complication associated with the administration of ephedrine has been reported in a recent publication by Valentine and Fitzgerald.[10] In Table I, three patients complaining of retention of urine from ephedrine were able to take large doses of propadrine hydrochloride without any evidence of urinary disturbance, and their asthmatic symptoms were relieved.

CONCLUSIONS

1. Propadrine hydrochloride in therapeutic doses may be administered freely without any evidence of nervousness or insomnia.

2. Propadrine hydrochloride, apparently, may safely be used in cases of hypertrophy of the prostate.

3. In children, propadrine hydrochloride does not produce restlessness or walking or talking in their sleep.

4. Tachycardia and palpitation, which have been fairly common symptoms associated with the administration of ephedrine, were not observed in this group of patients.

5. Propadrine hydrochloride has proved a very satisfactory and valuable therapeutic agent in the treatment of allergic manifestations.

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THE RELATION OF AGE AND SEX TO INCIDENCE AND PROGNOSIS IN PHENYLCINCHONINIC HYPERSENSITIVITY

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PLAINFIELD, N. J.

ALTHOUGH the question of phenylcinchoninic hypersensitivity has received wide attention in the recent medical literature, little study has been made of the many factors affecting the incidence and outcome of such cases. Some of these factors have been studied previously by the author.[1, 2] The paucity of literature on the incidence and prognosis of idiopathic acute yellow atrophy of the liver is remarkable.

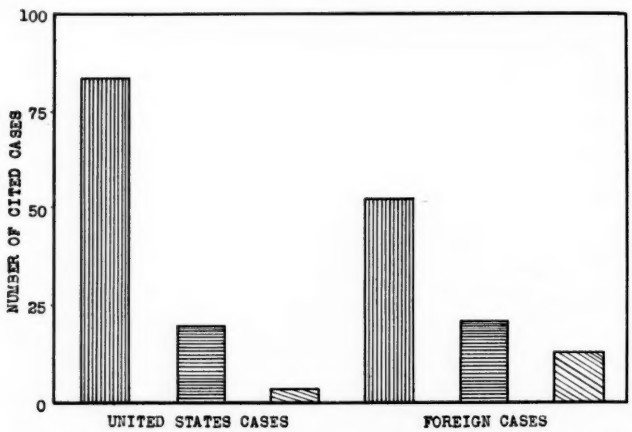

Fig. 1.—The relation of American and foreign age groups, by thirds of the population, to the incidence of phenylcinchoninic hypersensitivity (1913 to 1935). Vertical bars, age group 41 and over; horizontal bars, age group 21 to 40; slanting bars, age group 0 to 20.

From a perpetual tabulation which the author keeps currently upon phenylcinchoninic toxicity, there has been compiled Table I, and from that Fig. 1, to show the incidence by years in the three age groups of the population that are approximately equal.[3] Of the 187 cases reported throughout the world from 1913 to 1935 inclusive, incomplete cases being omitted, 15, or 8 per cent, occurred in the lower-third population group (age 0 to 20 years); 38, or 21 per cent, occurred in the middle-third population group (age 21 to 40 years); and 134, or 71 per cent, occurred in the upper-third population group (age 41 and over). Since it is probably not true that persons 41 years of age or over are more prone to febrile arthritic diseases than those from 21 to 40 years of age, it is obvious that the incidence of phenylcinchoninic hypersensitivity increases with age. It would appear that the lowest

group above cited has least chance of intoxication; the middle group is intermediate in risk of intoxication; and the oldest group stands the greatest chance of intoxication.

TABLE I

THE RELATION OF AMERICAN AND FOREIGN AGE GROUPS, BY THIRDS OF THE POPULATION, TO THE INCIDENCE OF PHENYLCINCHONINIC HYPERSENSITIVITY
187 Cases; Incomplete Cases Omitted

YEARS	AGE GROUPS					
	0-20		21-40		41 and over	
	U. S.	FOREIGN	U. S.	FOREIGN	U. S.	FOREIGN
1913	0	0	0	1	0	5
1914	0	0	0	0	0	0
1915	0	0	0	2	0	2
1922	0	1	0	3	0	5
1923	0	0	0	0	0	1
1924	0	0	0	0	3	0
1925	0	0	0	0	1	0
1926	0	0	0	1	0	6
1927	0	0	0	4	0	6
1928	0	0	1	0	0	5
1929	1	0	0	2	3	2
1930	0	9	4	1	5	2
1931	1	1	4	2	11	7
1932	1	0	2	1	18	2
1933	0	0	1	2	20	3
1934	0	0	5	1	20	2
1935	0	1	1	0	2	3
Totals	3	12	18	20	83	51
Per cent	2	6	10	11	44	27

TABLE II

THE RELATION OF AGE GROUPS, BY THIRDS OF THE POPULATION, TO THE FATALITY OF PHENYLCINCHONINIC HYPERSENSITIVITY

190 Cases; Incomplete Cases Omitted

YEARS	AGE GROUPS					
	0-20		21-40		41 AND OVER	
	FATAL	NONFATAL	FATAL	NONFATAL	FATAL	NONFATAL
1913	0	0	0	1	0	5
1914	0	0	0	0	0	0
1915	0	0	0	2	0	2
1922	0	1	1	2	0	5
1923	0	0	0	0	0	1
1924	0	0	0	0	0	3
1925	0	0	0	0	1	0
1926	0	0	1	0	3	3
1927	0	0	1	3	2	4
1928	0	0	1	0	4	1
1929	1	0	0	2	2	3
1930	0	9	2	3	2	5
1931	2	0	3	3	14	4
1932	1	0	1	2	13	7
1933	0	0	1	2	10	13
1934	0	0	2	5	12	12
1935	1	0	1	0	1	4
Totals	5	10	14	25	64	72
Per cent	2.6	5.3	7.4	13	34	38

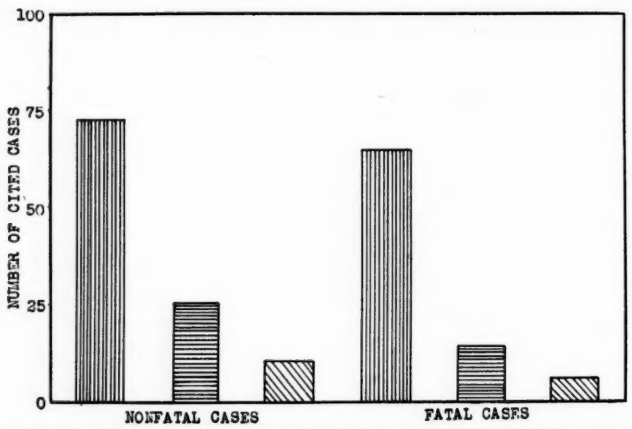

Fig. 2.—The relation of age groups, by thirds of the population, to the fatality of phenylcinchoninic cases. Vertical bars, age group 41 and over; horizontal bars, age group 21 to 40; slanting bars, age group 0 to 20.

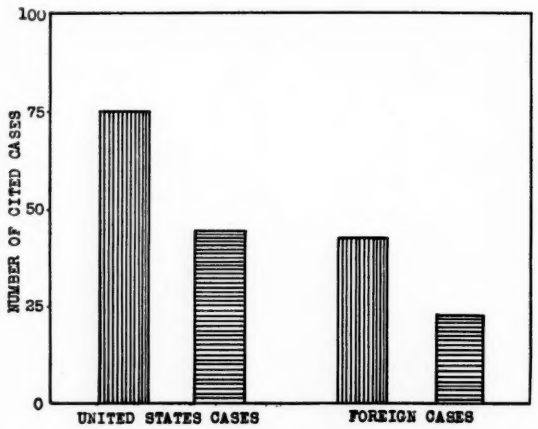

Fig. 3.—The relation of sex and location to the incidence of phenylcinchoninic hypersensitivity. Vertical bars, females; horizontal bars, males.

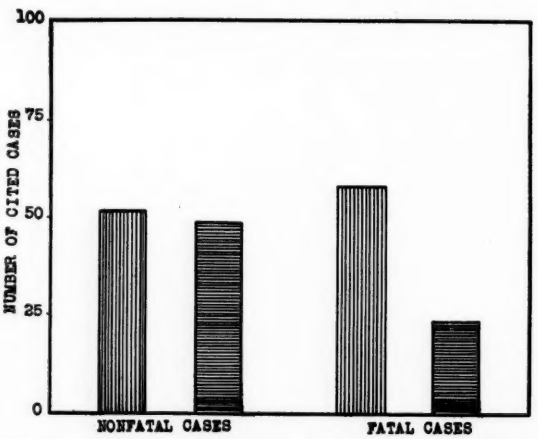

Fig. 4.—The relation of sex to the fatality of phenylcinchoninic hypersensitivity. Vertical bars, females; horizontal bars, males.

The relation of fatality to nonfatality in the three age groups above described is shown in Table II and Fig. 2. Of the 190 cases reported, incomplete cases being omitted, 2.6 per cent resulted fatally, and 5.3 per cent nonfatally, in the lower group (0 to 20 years of age); 7.4 per cent and 13 per cent were, respectively, fatal and nonfatal in the mid-

TABLE III

THE RELATION OF SEX, ARRANGED BY AMERICAN AND FOREIGN CASES, TO THE INCIDENCE OF PHENYLCINCHONINIC HYPERSENSITIVITY

185 Cases; Incomplete Cases Omitted

	MALES			FEMALES		
YEARS	U. S.		FOREIGN	U. S.		FOREIGN
1913	2		0	3		1
1914	0		0	0		0
1915	0		1	0		3
1922	0		7	0		3
1923	0		1	0		0
1924	2		0	1		0
1925	1		0	0		0
1926	0		1	0		6
1927	0		0	6		5
1928	0		1	1		4
1929	1		0	3		4
1930	3		2	6		1
1931	4		6	12		4
1932	13		1	9		2
1933	8		1	13		5
1934	9		1	19		2
1935	1		2	2		2
Totals	44	68	24	75	117	42
Per cent		36.7			63.3	

TABLE IV

THE RELATION OF SEX TO THE FATALITY OF PHENYLCINCHONINIC HYPERSENSITIVITY

184 Cases; Incomplete Cases Omitted

	MALES		FEMALES	
YEARS	FATAL	NONFATAL	FATAL	NONFATAL
1913	0	2	0	4
1914	0	0	0	0
1915	0	1	0	3
1922	1	6	0	3
1923	0	1	0	0
1924	0	2	0	1
1925	1	0	0	0
1926	1	0	3	3
1927	2	4	1	4
1928	1	0	4	1
1929	0	1	3	4
1930	1	3	3	4
1931	5	5	14	2
1932	8	6	8	3
1933	1	8	10	8
1934	2	8	12	9
1935	1	2	2	2
Totals	24	49	60	51
Per cent	13	26	32	28

dle group (21 to 40 years of age); and 34 per cent and 38 per cent were, respectively, fatal and nonfatal in the upper group (41 years and over).

The effect of sex upon incidence and outcome of phenylcinchoninic hypersensitivity is quite striking. Of the 185 cases, incomplete cases being omitted, here reviewed, 68, or 36.7 per cent, were males; and 117, or 63.3 per cent, were females. Table III and Fig. 3 show the incidence by years. Apparently there is a definite preponderance of liability to this condition among females over males. The author is inclined to believe that this tendency should not be overemphasized, since more females undoubtedly receive analgesic medication than males. This may not, therefore, be a true sex difference at all.

Table IV and Fig. 4 are arranged to show the relation of sex to survival in such cases of hypersensitivity. Of the 73 males in this series (184 cases), 24, or 30 per cent, died; and 49, or 70 per cent, recovered. Among the 111 females in this series, 60, or about 54 per cent, died; and 51, or about 46 per cent, recovered. These figures appear to indicate that expectation of recovery is greater in males than in females.

SUMMARY

1. Cases of phenylcinchoninic hypersensitivity throughout the world are classified by age groups, from which it appears that incidence rises with increasing age.

2. The prognosis is probably independent of age, but dependent upon whether or not the primary symptoms are hepatic or superficially allergic. The published data are still too incomplete to warrant any conclusion.

3. Cases of phenylcinchoninic hypersensitivity throughout the world are classified by sex, from which it appears likely that the incidence is higher in females than in males; and that expectation of recovery is greater in males than in females.

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Special Reports

ECZEMATOUS DERMATITIS DUE TO CHLORAL HYDRATE

(Following Both Oral Administration and Topical Application)

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WHILE eczematous contact-type dermatitis due to chloral hydrate is not of frequent occurrence, several proved cases have been recorded. In 1933, Abramowitz and Noun[1] reported such a case and reviewed the older literature. Since this report, two more cases have been described, the first by Tulipan[2] and the second by Flandin, Rabeau, and Ukrainczyk.[2] Tulipan's case was of maculopapular and squamous character and occurred after peroral use of the drug. However, a patch test with 5 per cent chloral hydrate gave a positive reaction, eliciting an eczematous response. Flandin's case presented a papulovesicular eruption with swelling on the upper lip and, to a lesser degree, on the lower lip. These manifestations appeared after peroral use of the drug. Here, too, the patient's skin reacted to a patch test with a 1 per cent solution of chloral hydrate, presenting a severe bullous eruption at the site of application.

We here present an additional case with the interesting features that the eczematous eruption occurred both after oral and topical exhibition of chloral hydrate; and that the hematogenous eruption was limited to the scalp, face, neck, hands, and forearms (a distribution resembling that of the recently reported hematogenous eruptions following sulfanilamide).

CASE REPORT

On our first examination, P. S., 71 years old, presented an acute eczematous, weeping exacerbation of a persistent lichenified eruption on the occipital part of the scalp and on the nape of the neck. He had during the past year been treated with various ointments which, the patient stated, did not improve the original condition but which, in some instances, produced marked irritations of the skin.

When first seen at our office, the patient presented a diffuse erythematous, edematous dermatitis of the upper neck, with oozing in the postauricular region and with a fairly sharply demarcated border at the lower margin, just below the collar line. The patient was given soothing local therapy in the form of wet dressings and bland ointments in addition to small doses of roentgen ray irradiation. He improved considerably under this regime and the acute dermatitis subsided. However, he continued to suffer from itching and sleeplessness at night for which condition he was given a small dose (0.36 c.c.) of chloral hydrate by mouth.

The day after taking this drug internally for the first time, he had a marked acute exacerbation of his eruption on the neck and scalp, as well as new lesions on the face, back of the hands and wrists. These lesions were of erythemato-squamous character on the face and hands, while the volar aspects of the wrists were covered with smaller than pinhead-sized flat-topped papules on a slightly erythematous base. This exacerbation of the dermatosis seemed to have some relationship to the use of chloral hydrate and the patient was patch tested with a 2.5 per cent solution of the drug, to which he gave a three-plus reaction with edema, erythema, and vesiculation (B. Bloch). On further investigation it was found that one of the ointments previously prescribed had contained 0.5 per cent chloral hydrate and that the application of this ointment had preceded the marked exacerbation for which the patient sought relief on his first visit. In addition to this positive reaction to chloral hydrate, the patient gave positive reactions to patch tests with calmitol, eucupine, benzocaine, resorcinol and lanolin, each of which had been present in one or the other of the many prescriptions previously given by other physicians.

COMMENT

The clinical course and the results of the patch tests demonstrate that this patient presents a very marked contact-type eczematous hypersensitivity to chloral hydrate and that the same type of cutaneous manifestations of this hypersensitivity were elicited both by local application of the substance and by hematogenous distribution to the skin after oral administration. This phenomenon has been previously observed by several others.[3-6] Our case confirms the opinion of Sulzberger and Kerr[6] that the skin of the scalp, face, hands, and forearms is a site of predilection for eruptions of this type (''hematogenous contact-type dermatitis''). The reasons for this predilection are unknown. Nevertheless, the possibility exists that the previous external contact of these particular areas with the substance in topical application may have produced in these sites a degree of skin sensitivity greater than that of the remaining skin areas.[6] It is noteworthy that in spite of the characteristic localization of the hematogenous dermatosis, there was, in our case, no evidence that photosensitization played a direct role, there having been no exposure to direct sunlight or to ultraviolet rays prior to the eruption.

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962 PARK AVENUE

ATOPIC DERMATITIS WITH RAPIDLY MATURING CATARACTS

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CATARACT associated with so-called neurodermatitis was first reported in this country by W. T. Davis in 1921, as cited by Daniel,[1] in her article reporting three cases. She refers also to the observation of this association by Rothmund, Andogsky, Lowenstein, Gault, and Kurz. Little attempt has been made to explain this syndrome, except to observe that both conditions represent changes in the ectodermal tissue. In discussing Dr. Daniel's cases, Dr. Alan C. Woods expressed the opinion that allergens in the aqueous humor, acting on the lens capsule, may increase the permeability of the capsule, and that the cataract is essentially traumatic.

We report this case to give additional evidence that the association is not coincidental, as some have thought, but is a definite syndrome with a common cause, and that the basis is atopy.

Without splitting hairs over the terms "atopic dermatitis" and "neurodermatitis," the authors are willing to accept either. We know that neurodermatitis frequently has an atopic basis, so do not confuse this condition with contact dermatitis, an entirely different phenomenon.

CASE REPORT

G. B., a white female, aged 15 years, came under our observation in August, 1936, having been referred by a dermatologist. Her complaint was eczema and loss of eyesight. An older sister had had a similar skin condition in early childhood, but had recovered spontaneously. The patient's skin trouble began at age 7 in the flexural areas, and gradually spread until practically all the skin was involved. Three months before we saw her she began to notice impairment of vision. The latter condition became evident after she had had three doses intravenously of what we believe, on the physician's word, to have been some calcium salt. Loss of vision was rapid and for the last three weeks she had been unable to see to go about by herself.

On physical examination she was well nourished, 64 inches in height, and weighed 118 pounds. With the exception of the scalp, palms, soles, and a small area high on the medial surfaces of each thigh, the skin was involved in a confluent maculopapular eruption, with variations. The face was less acutely affected than the trunk and extremities. The skin was thick and appeared edematous. There was much evidence of pruritus, with fissuring in the cubital and popliteal areas and on the anterior surface of the neck.

Vision in both eyes was limited to the detection of limited objects. The palpebral conjunctivae were thickened but not granular. The pupils were equal and active, irises normal, and corneas clear. Both lenses were opaque and the fundi could not be seen. Light projection was good.

On attempting to make skin tests on the least inflamed areas of the back, the epidermis was found to be hardened, suggesting a thin coating of shellac. It was so resistant to intradermal testing that only scratch tests could be made directly. These were followed by passive transfer tests on a person with history negative for atopy. The following reactions were observed:

Scratch tests:

Silk ++++	Peach +
Irish potato +++	Orange +
	Goat hair ++

Passive transfer tests:

House dust +	Silk +++	Grapefruit +
Bermuda pollen +	Goat hair +	Blackberry +
Lemon +	Bean +	Peach +
Peanut +	Tomato +	Irish potato +
Pecan +	Orange +	Watermelon +

The laboratory findings were all within normal limits. The blood eosinophiles were 4 per cent.

The cataracts were operated upon, and with her correction the vision is 20/20 in each eye. She is in school and drives a car.

Treatment of the dermatitis has been directed primarily against the atopic factors, and has been somewhat of a matter of trial and error. Silk avoidance has been incomplete, as the sister is a dancing instructor and the mother works in a ready-to-wear establishment. Improvement has, however, been steady from the first, although retarded by an occasional acute flareup, the cause of which has usually been known. Silk desensitization was attempted but after several doses pruritus would become worse for a day or two after treatments. This was stopped but we believe it was beneficial.

Instructions were given on house dust avoidance and improvement has been continuous. At this time the skin is approaching normal.

The patient found that inhalation of silk dust, or ingestion of potatoes or wheat would aggravate the pruritus. In this regard it was interesting to note that the receptor of the allergic serum would have intense itching of her back with wheal formation at the sites of the serum injections when she handled an unusual amount of silk. After she volunteered this information she was asked to note any effect from eating potatoes. She could detect none. The passive sensitivity lasted about three weeks.

REFERENCE

1. Daniel, Ruby K.: Allergy and Cataracts, J. A. M. A. **105**: 481, 1935.

Society Proceedings

SOCIETY FOR THE STUDY OF ASTHMA AND ALLIED CONDITIONS

ANNUAL MEETING, ATLANTIC CITY, N. J., APRIL 30, 1938

Resolutions

WHEREAS, the Society for the Study of Asthma and Allied Conditions has received the sad news of the death of Dr. Henry W. Straus, and

WHEREAS, the Society feels deeply that a great loss has been sustained by the members themselves personally and by the whole profession, and

WHEREAS, his personality, character, and affability were superlative in quality, and

WHEREAS, his contributions to the field of Immunology and Pediatrics were of signal importance, be it

Resolved, That we, the Society for the Study of Asthma and Allied Conditions, formally and heartfully register our poignant sense of loss in the passing of this man, and be it

Resolved, That a copy of these resolutions be spread upon the minutes of the Society, that a copy of them be forwarded to his wife, and that a notice be printed in the JOURNAL OF ALLERGY.

CHICAGO SOCIETY OF ALLERGY

CHICAGO, ILL., APRIL 18, 1938

The Inhalation of House Dust and Horse Dander as an Etiological Factor in Atopic Dermatitis. SAMUEL J. ZAKON, M.D., AND SAMUEL J. TAUB, M.D. (Abstract.)

The patient, a male 28 years of age, had a generalized eczema with marked lichenification of the skin, of sixteen years' duration, on the face, neck, hands, and flexures of the elbows and knees. Intradermal tests were positive to autogenous house dust extract and horse dander, and negative to foods.

The Prausnitz-Küstner reaction showed a high reagin titer to dust and horse dander. A small amount of powdered horse dander blown into one nostril was followed by symptoms of coryza and aggravation of the eczema within twelve hours. Itching, especially, was aggravated.

In the treatment, dust precaution and the wearing of a mask which covered the entire head and neck at night plus desensitization to autogenous dust and horse dander completely relieved the itching and eczema. It would seem that complete environmental control is essential in the management of some cases of allergic eczema.

The Effect of Volume on Absorption of Antigens. THEODORE B. BERNSTEIN, M.D., S. S. RUBEN, M.D., AND SAMUEL M. FEINBERG, M.D. (Abstract.)

The rate and degree of absorption of ragweed extracts differing in volume, but of the same antigenicity, were measured and compared in a group of nonallergic subjects, using a method which has been described previously (Feinberg, S. M., and Bernstein, T. B.: A Method of Measuring Rate and Degree of Absorption of Antigens, J. ALLERGY **8**: 523, 1937). The extracts used in these experiments were 0.2 c.c. of a 3 per cent and 2.0 c.c. of a 0.3 per cent mixed ragweed. The small concentrated volume of 3 per cent ragweed extract was injected subcutaneously into a subject whose forearm had twenty-four hours before been intradermally sensitized at eight sites with serial dilutions of ragweed serum. After several minutes, reactions began to appear at the sites. The appearance time of the first reaction, the time at which the last reaction took place, the number of sites reacting, and the degree of reaction and reagin titer at each of the sites reacting was determined. The same individual was then resensitized forty-eight hours later, and the effect of an injection of the large volume of 0.3 per cent ragweed extract upon the time, rate, and degree of reaction was noted.

In 10 of the 11 subjects, reactions were observed sooner at the various serum sites, and in 8 of these 10 patients, sites of higher serum dilutions reacted when small volume injections were given. Although all (13) of these higher serum dilution sites reacted within sixty-one minutes, only 2 of the corresponding sites had reacted in that time following large volume administration.

In the majority of subjects, both the first demonstrable and the maximum amounts of antigen in the circulation occur earlier, and the height of the titer in the circulation is greater with the small volume; this indicates that constitutional reactions in the treatment of hay fever are apt to occur more frequently and earlier when small concentrated volumes are injected.

Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL FEINBERG, M.D., CHICAGO

The Function of the Bronchial Tubes. Ellis, M.: Lancet **1:** 734, 1938.

The author made a number of studies of bronchial function in dogs by means of a rubber balloon placed in a bronchus and connected to a pen and recording apparatus. The graphs clearly show that the bronchial lumen widens during the inspiratory phase and narrows during the expiratory phase of respiration. The mechanism of these rhythmic changes was shown to be entirely due to the mechanical movements of the chest wall.

Many factors were shown to modify this normal bronchial tone. For example, adrenalin produced decided dilatation of the bronchus in the normal animal. Various chemical and other manipulations within the nose resulted in an increased tone of the bronchi. It is the opinion of the author that the presence of viscid sputum acts as an irritant, increasing reflexly the tonicity of bronchi. The author emphasizes the fact that asthma is in reality an increased tonicity of sufficient extent to interfere with exchange of air.

The Specific Treatment of Hay Fever and Pollen Asthma in Denver. Johnson, R. P.: Rocky Mountain M. J. **35:** 305, 1938.

According to Johnson, the pollinating season in Denver is from March to October. During March, April, and May the tree pollens cause hay fever. Of these, the cottonwood is most important. The author believes that some of the cottonwood cases are sensitive also to the "cotton" as well as to the pollen, and, therefore, they may have symptoms after the pollinating season. The grass season occurs from May through July and is caused principally by June grass, orchard grass, timothy, wheat grass, and red top. The weed season is the most important and has a spread from July to October. The chief offenders are Russian thistle, sages, ragweed, and pigweed. The author believes that the scratch test is the method of choice in diagnosis.

Emergency Treatment in Asthma (Asthmatic Crisis). Waldbott, G. L.: J. A. M. A. **110:** 1423, 1938.

The author considers an asthmatic crisis as a type of allergic shock in which the lungs are the shock organs. He outlines the management of the asthmatic crisis along four lines: 1. Avoidance of harmful antigens. He cautions against the use of many drugs to which the individual may be sensitive. He finds that foods that had not been ingested for a long time are particularly harmful. 2. Elimination of antigens is regarded as an important step. A dust-free room is advisable. He believes that adrenalin should be usually discontinued. 3. Symptomatic treatment consists usually of sedation, primarily by the use of barbiturates, although occasionally pantopon may be necessary. Helium with oxygen may be used to advantage at times. Iodides are helpful. Intravenous injections of hypertonic dextrose solutions are commonly used. Frequently the author em-

ploys blood transfusions. In cases where other methods fail bronchoscopic aspiration is recommended. 4. Rapid hyposensitization with antigens to which the patient gives a skin reaction or to which he is exposed at the time of the attack is regarded by Waldbott as an important step in the alleviation of the asthmatic crisis.

Roentgen Studies of the Pathological Physiology of Bronchial Asthma. Rigler, L. G., and Koucky, R.: Am. J. Roentgenol. **39**: 353, 1938.

The authors made a roentgenologic study of the bronchi of asthmatic patients by means of iodized oil. They believe that such studies throw considerable light on the pathology and physiology of asthma. In early cases the caliber of the bronchi is distinctly narrowed. Later some of the bronchi fail to show the iodized oil shadows, indicating that occlusion has taken place. The presence of occlusive plugs can be definitely shown by this procedure. From this bronchographic and pathologic study the authors conceive the mechanism of asthma to be as follows: The first effect is a hypersecretion of mucus which accumulates within the lumina of the bronchi. Stasis results possibly due to spasm of the bronchial muscle or to marked viscosity of the mucus. A plug is formed partially obstructing the bronchus so that air may be inspired past the occlusion, but is expelled less readily. Emphysema is thus produced. During the asthmatic attack the spastic bronchial muscle clamps down around this plug, aggravating the occlusion.

Dermatology

UNDER THE DIRECTION OF MARION B. SULZBERGER, M.D., NEW YORK CITY, AND JOSEPH GOODMAN, M.D., BOSTON

Eczematous Eruptions Produced by Leaves of Trees and Bushes. Genner, V., and Bonnevie, P.: Arch. Dermat. & Syph. **37**: 583, 1938.

This report concerns three cases of eczema of the face and hands, proved, by studies which included careful investigation by patch testing, to be caused in two instances by elm leaves and in one instance by magnolia leaves. The authors point out that in the study of contact dermatitis little attention has been paid in the past to the leaves of trees and bushes, although the part played by barks and woods has been the subject of considerable attention.

Dermatitis Due to Cocobolo Wood. Abramowitz, E. W., and Swarts, W. B.: Arch. Dermat. & Syph. **37**: 441, 1938.

The authors report a patient with a dermatitis of four months' duration, limited to the face, neck, scalp, arms, and the posterior aspect of the knees. The onset of the eruption occurred when the patient started to carve a piece of cocobolo wood. Patch tests with dry cocobolo sawdust or with an ether extract of the wood were positive on three occasions. After each of these patch tests, there was a general flare-up of the eruption.

The Value of the Patch Test in the Investigation of Occupational Dermatitis. Peters, M.: Dermat. Wchnschr. **106**: 50, 1938.

The author presents statistically her experience with the use of the patch test in the investigation of 100 cases of dermatitis of possible industrial origin. In

54 instances patch tests supported a diagnosis which could be made with fair certainty from the history of the patient. Forty-six of these cases were found to be industrial in origin, 8 nonindustrial. In 40 further instances (14 industrial and 26 nonindustrial) the patch test was the most important factor in establishing the correct diagnosis. In 5 instances, in spite of the absence of patch test reactions, the history of recurrence of the eczema on re-exposure to occupational factors enabled the author to establish a diagnosis of industrial dermatitis. In the remaining one case of the 100 cases no decision as to the industrial or nonindustrial nature of the dermatosis was possible.

The author concludes that the patient's history, the physical examination, and the patch test reactions must be accorded equal importance in the study of possible industrial dematitis.

Cutaneous Hazards in the Citrus Fruit Industry. Schwartz, L.: Arch. Dermat. & Syph. **37**: 631, 1938.

A complete review of the citrus fruit industry revealed that the cutaneous hazards, apart from trauma, are as follows: in fruit growers, dermatitis from fertilizers and insecticides; in packers, dermatitis from washing solutions and from citrus oils to which the packers may become hypersensitive; in canners, dermatitis and paronychia due to hypersensitivity to citrus oils and juices. No cases of dermatitis were encountered which could be attributed to dyes used to color oranges. Likewise the author found no dermatologic diseases which could be attributed to "precoloring" fruit with ethylene or nitrogen trichloride.

An extensive study of the patch test reactions to various citrus fruit juices, peels, and extracts revealed that most skins did not react to a patch test with pure fruit juice left on for twenty-four hours. However, further experiments indicated that the oil contained in the peel of the citrus fruit was a primary irritant substance when applied as a patch test. The peel itself presented no primary irritant qualities unless it was broken or crushed before application to the skin, to permit the escape of the oil.

Contact Dermatitis. Curtis, G. H.: M. Clin. North America **22**: 371, 1938.

The author presents a general discussion of contact dermatitis, in which he gives an excellent summary of present knowledge of this common dermatosis. For the sake of clarifying diagnostic procedures the author divides into three groups the clues or leads to the discovery of the substance producing the contact dermatitis. These three groups are:

I. Clues or leads of a general nature relative to the environment, leading to the discovery of the cause of a disseminated dermatitis of the exposed areas. Examples of such clues are periodic or seasonal exacerbations, flares of the dermatitis which can be related to environmental factors, hobbies, occupational exposures, etc.

II. Clues or leads suggesting certain causal agents which produce a dermatitis on certain local areas of the exposed surfaces. Examples of such clues are dermatitis of the forehead from hat bands; of the face and scalp from hair tonics and hair dyes; of the lips from lipstick; of the neck from fur collars, jewelry, etc.

III. Leads or clues leading to the discovery of the irritants usually affecting the covered areas of the cutaneous surface. Examples are dermatitis of the axillae from dress shields, depilatories or deodorants; of the genitalia from douches or suppositories, as well as from many allergens transferred from the fingers; the feet and ankles from socks, shoes, etc.

The paper further includes a discussion of the diagnosis, differential diagnosis, and treatment of contact dermatitis.

Atopic Dermatitis. Netherton, E. W.: M. Clin. North America **22**: 333, 1938.

The author presents a general review of the problem of atopic dermatitis, discussing among other things the constitutional, allergic background of this disease as well as the neurocirculatory factors. There is an excellent review of skin testing, elimination procedures, and treatment in general.

Statistics derived from 50 selected cases of atopic dermatitis studied by the author showed that the average age was 19.5 years. There was a family history of allergy in 33 cases. There was a family history of eczema on the side of one parent in 15 cases, on the side of both parents in only one case. There was a past history of infantile eczema in 37 patients, and there were associated allergies (usually asthma or hay fever) in 27. All of the patients gave positive scratch-test reactions to many foods, and 48 of the 50 patients gave a positive scratch-test reaction to one or more inhalant allergens. Twenty-four patients reacted to milk, 26 to egg, and 22 to wheat. The highest number of positive scratch-test reactions to food proteins in a single case was 53. House dust, silk, orris root, and feathers were the inhalant allergens which produced the greatest number of positive scratch-test reactions.

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., BROOKLYN.

The Conjugation of Haptens in Vivo. 1. Phenolphthalein. Rosenthal, S. R.: J. Immunol. **34**: 251, 1938.

The author reports his work on attempts to sensitize guinea pigs and rabbits to phenolphthalein.

In early experiments, the simple injection of colloidal forms of phenolphthalein, made with gelatin or human serum, failed to incite either cutaneous or precipitative reactions, notwithstanding the sensitivity produced by the human serum. Having observed that parenterally administered phenolphthalein appeared in the blood in both free and conjugated forms, the author continued his experiments, using as an antigen a form of phenolphthalein conjugated in an animal.

Animals (guinea pig, rabbit, cat) were fed phenolphthalein or injected with colloidal phenolphthalein. The serums obtained from these animals were used as antigens to inject into other animals of the same or of different species. The writer was then able to demonstrate skin sensitivity to both colloidal and body-conjugated phenolphthalein in the animals treated. The animal receiving the phenolphthalein orally or by injection demonstrated no sensitivity to the drug. Conjugation of phenolphthalein with serum could not be accomplished in vitro.

Despite the fact that anaphylactic shock could not be produced and precipitins did not appear, the author is of the opinion that the reaction produced to phenolphthalein is allergic in nature and that by this method other nonantigenic substances may be made antigenic.

Studies on Inflammation. XIV. Isolation of the Factor Concerned With Increased Capillary Permeability in Injury. Menkin, V.: J. Exper. Med. **37**: 129, 1938.

Previous studies by the author have indicated the presence, in various types of exudates, of a factor, which, when brought into contact with normal capillaries, induces a prompt increase in filtration through the capillary endothelial wall.

The writer has been unable to obtain concrete evidence to support the view that a histamine-like substance exists in exudates, or in their partially purified active fraction.

These studies have been continued in the present communication. A method for the isolation and purification of this permeability factor is described.

The purified fraction obtained from exudate was found to be a crystalline, double refractive, nitrogenous substance, which is not a protein, yet contains amino and carboxyl groups. The active material is dialyzable and is precipitated by concentrated ammonium sulfate. On the basis of the experimental evidence presented, the author is of the opinion that the active substance is an intermediary breakdown product of protein metabolism, probably belonging to the group of relatively simple polypeptides.

The Fate of Diphtheria Toxin Injected Into the Subcutaneous Tissue of the Guinea Pig. D'Antona, D.: Rev. d'Immunol. **3**: 413, 1937.

The author reports studies on the fate of diphtheria toxin injected subcutaneously into the guinea pig. One M.L.D. of diphtheria toxin was injected subcutaneously into guinea pigs. The serofibrinous exudate, which formed locally, was removed at various intervals and tested for toxicity on the rabbit.

The author observed that the serofibrinous exudate, formed locally after the injection of diphtheria toxin, exhibited toxic properties up to sixty hours after injection. This toxic phase was followed by a neutral phase, during which the exudate was not toxic. During the third or antitoxic phase the exudate showed definite antitoxic properties. Guinea pigs immunized with formol toxoid showed a shortened toxic phase and an accelerated antitoxic phase.

The Rate of Disappearance of Crystalline Egg Albumin Following Its Intravenous Injection. Kenton, H. B.: J. Infect. Dis. **62**: 48, 1938.

The author reports data concerning the rate of disappearance of crystalline egg albumen introduced into the blood stream of rabbits.

Ten cubic centimeters of a 1 per cent solution of crystalline egg albumen was injected intravenously into each of 16 rabbits and blood samples were drawn at intervals thereafter. The relative amounts of egg albumen present were determined by precipitin titrations.

The results of the study indicated that 90 per cent of the total amount of egg albumen in the blood stream had disappeared from the circulation within the first hour after injection; 96 per cent within two hours, and 99.76 per cent within twenty-four hours. The writer notes that this rapid rate of disappearance of crystalline egg albumen did not apply to other proteins, which seem to vary in their periods of retention in the blood stream.

Microscopic Observations on the Behavior of Living Blood Vessels of the Rabbit During the Reaction of Anaphylaxis. Abell, R. G., and Schenck, H. P.: J. Immunol. **34**: 195, 1938.

The authors report their observations on the behavior of the arterioles, capillaries, and venules in the normal rabbit and in the same rabbit later sensitized and injected with the anaphylactogen.

The technique evolved to make these observations feasible is described in detail. The studies were made with the high power of the microscope upon the arterioles, capillaries, and venules of thin layers of living tissue in a transparent moat chamber ingeniously introduced into the rabbit's ear.

The rabbits were sensitized with horse serum and the vessels observed after the intravenous injection of the antigen and after direct introduction of the antigen into the moat of the chamber. Following the intravenous or local injection of the antigen, the authors observed arteriolar contraction, increased adherence of leucocytes to the vascular endothelium, leucocyte emigration, and the formation of leucocytic clumps, which on occasion blocked the circulation. No contraction of the capillaries or venules was noted, nor was any specific precipitate observed at any time in the blood vessels.

Skin Reactions. I. The Effect of Allergic and Histamine Wheals on the Rate of Absorption of Dyes and Blood From the Human Cutis. Abramson, H. A., and Engel, M.: J. Investigative Dermat. **1**: 65, 1938.

The authors report their investigations on the effect of allergic and histamine wheals on the rate of absorption of crystalloidal and colloidal dyes, red blood cells, and blood pigment from the human dermis.

The crystalloidal dyes, phenol red, and bromthymol blue, and the colloidal dyes, trypan blue and Congo red were used in a 0.1 per cent concentration. Injections were usually made intracutaneously on the anterior aspects of the forearm or thigh of normal and ragweed sensitive individuals. When the wheals were produced by ragweed, the dye and ragweed were mixed prior to injection. Histamine (1:1,000) was either mixed with the dye and injected or introduced separately by iontophoresis.

Depots of both crystalloidal and colloidal dyes showed an accelerated absorption from the site of injection following the production of ragweed or histamine wheals in these areas. Attempts to reproduce, by means of simple mechanical processes, an effect similar to that resulting from specific whealing with ragweed or with histamine were essentially unsuccessful. The dye located in the erythematous areas of both the ragweed and histamine wheals did not show a speed of disappearance comparable to that observed in the area of the wheal itself. The size of the wheal was proportionate to the rate of disappearance of the dye.

Whole blood and some blood pigments, intracutaneously injected, underwent the same phenomenon of accelerated absorption. Histamine iontophoresis resulted in rapid absorption of extravasated blood.

No evidence of fixation of dyes by wheal formation was observed by the authors.

Pediatrics

UNDER THE DIRECTION OF WILLIAM C. DEAMER, M.D., NEW YORK

The Antitoxic Response to Diphtheric Antigens in Children. Fraser, D., and Halpern, K.: J. Immunol. **33**: 323, 1937.

The authors point out the limitations of the Schick test in determining the response to diphtheric antigens. It is qualitative rather than quantitative, and since it acts as a secondary stimulus, it invalidates subsequent comparison of the original antigenic materials. In this study of 244 children, from five to sixteen years of age, the antitoxin titer of their blood was determined on about 600 samples following administration of three doses of ordinary toxoid. All were Schick positive at the time of the first injection of toxoid. It was shown that the antitoxin level three months after three doses of toxoid compared favorably with that of naturally immune persons, but that in contrast with the latter it faded with time. The tabulated

results are suggested as a yardstick for assessing the relative value of any given diphtheric antigen. Comparisons are made by this method between alumn toxoid and regular toxoid in different dosages.

Hypertonic Dextrose Solutions in the Treatment of Bronchial Asthma. Stoesser, A. V., and Cook, M. M.: Journal-Lancet **58**: 12, 1938.

This report deals with the treatment of chronic epinephrine-resistant asthma in three children, 10 to 12 years old. Twenty-five per cent intravenous dextrose in amounts of 200 c.c. benefited one child with food allergy markedly. Oral dextrose was also effective in preventing attacks, whereas hypertonic saline was without benefit. The other two children received considerably less benefit from the intravenous dextrose, and this only when it was prepared with distilled water rather than saline. These were chiefly inhalant cases. They responded well to dietary salt restriction and asthma could be produced again by adding small amounts of salt to the diet. Both became cyanotic and had severe asthma when 25 per cent sucrose was used instead of dextrose.

The authors suggest that the variation in response to dextrose, depending on the etiology of the asthma, may open a new avenue of approach to the study of the underlying mechanism in allergic disease. They feel that asthma due to ingestants may be easily controlled by intravenous dextrose, but that pollen asthma is very resistant. When carbohydrate therapy fails to give satisfactory response, shifts in sodium chloride intake in the diet appear to influence the number and severity of attacks.

Concerning the Unsaturated Fatty Acid Content of the Serum Lipids in Infantile Eczema. Schornstein, L.: Ztschr. f. Kinderh. **59**: 52, 1937.

In a series of 26 eczematous and 25 normal infants, the total serum lipids did not differ, but the iodine number averaged 118 in the eczema group and 150 in the normals. To determine what effect the fat and salt-poor and carbohydrate and plant protein-rich diet of the eczema cases might have, 9 normals were placed on this diet. No significant effect was observed, but the diet was given for only three or four days. Two control cases fed a cod-liver oil preparation rich in unsaturated fatty acids showed marked rises in iodine number after three weeks. The study of the therapeutic effect of unsaturated fatty acids on eczema is still in progress.

The author believes the results reported may indicate secondary rather than causative changes in infants with eczema.

Prevention of Dermatitis Venenata Due to Poison Ivy in Children. Molitch, M., and Poliakoff, S.: Arch. Dermat. & Syph. **36**: 1086, 1937.

This is the second report on this subject by the authors, the first having appeared in a previous issue of the same journal. Forty-two children, all of whom had had poison ivy dermatitis within the previous ten weeks, were given midseason injections of an alcoholic extract of the plant hypodermically. Progressively larger amounts, diluted in distilled water, were administered at weekly intervals. The only reaction was a local one, consisting in mild itching for a few hours. The only child in the group to develop poison ivy dermatitis did so a few days after the first injection. Eight control children, not treated, all developed further attacks.

Concerning Generalized Vaccinia in Eczema. Naumann, O.: Kinderärztl. Praxis. **9**: 99, 1938.

The author believes that an infant who has eczema is predisposed, by pathologic changes in his blood vessels, to a hematogenous dissemination of the virus of vaccinia —quite apart from the question of surface dissemination by direct contact. This is

also the concept of Gins, Ellis, and others. A case of eczema vaccination is cited in which the child died with symptoms suggesting encephalitis. The child, 1½ years old, developed the rash on the eighth day at about the height of the reaction at the primary vaccination which had been done when the child was practically free of eczema. The close identity of eczema vaccination and generalized vaccinia is emphasized.

Accidental Smallpox Vaccination and Eczema Vaccination. Graves, G. W., and Dowman, C.: New York State J. Med. **37**: 1833, 1937.

The literature on generalized vaccinia and eczema vaccination is reviewed briefly and the predisposition of the eczematous child to hematogenous spread is pointed out as in the previous article. Two fatal cases are reported—both originating from a sibling who had been vaccinated.

Miscellaneous

Acquired Sensitivity to Cinchophen. A Report of Six Cases and a Review. Sugg, E. S.: Am. J. M. Sc. **195**: 473, 1938.

Thirty-two cases of cinchophen poisoning are reported, including six by the author, which appear to illustrate the phenomenon of an acquired sensitivity to this drug. The case histories are divided into two groups: a cutaneous group of 10 cases and 2 deaths, and a hepatic group of 22 cases and 12 deaths. Many theories have been advanced as to the exact cause of the toxicity of cinchophen, only two of which are discussed. One is that the necrosis of the liver is due to an abnormal susceptibility of the individual because of certain predisposing conditions; the other deals with the theory that the various toxic manifestations of cinchophen are allergic in nature. An individual may not show toxic symptoms from the use of cinchophen upon its first administration, yet damage may have occurred which is accentuated by readministration even after several months or years. The mechanism of acquired sensitivity from nonprotein substances such as cinchophen may be explained by Landsteiner's demonstration of a combination of the sensitizing substance with the body proteins to form an antigen.

Ergonovine Versus Ergotamine as a Terminator of Migraine Headaches. Lennox, W. C.: Am. J. M. Sc. **195**: 458, 1938.

Ergonovine is a new and more active oxytocic alkaloid of ergot. It has been used in 78 patients suffering from attacks of migraine. The drug is put out under the trade names of "Ergobasine tartrate," "Neo-gynergen," "Ergoklonin," "Ergometrine," and "Ergotrate" by various pharmaceutical concerns. Its injection terminated headache in 37 per cent against 89 per cent of patients receiving injections of ergotamine tartrate. It brought partial or temporary relief to an additional 40 per cent of the patients. The proportion not helped at all after ergonovine was 21 per cent and after ergotamine, 6 per cent.

The Treatment of Migraine With a Carbohydrate-Poor Diet. Porges, S.: Med. Klin. **33**: 664, 1927.

The author has treated numerous cases with a diet poor in carbohydrate, several of whom had previously been on a salt-poor diet without satisfactory results. In some cases sugars and starches both were eliminated as well as salt; in others, carbohydrates alone were reduced. The best results were obtained in the former group.

Porges kept his patients on 50 to 100 grams of carbohydrates a day. This represented the equivalent of 100 to 200 grams of rye bread. The patients judged the amount to be taken by their symptoms, and sugar was gradually added. On re-establishing a normal diet migraine returned in some instances, and relief was obtained only so long as the diet was observed. This had to be constantly and rigidly enforced in the severe cases of "status migranosus."

Anaphylactic Reactions Following Medication With Parenteral Liver Extract. Kranz, C. I.: J. A. M. A. **110:** 802, 1938.

Allergic reactions from liver therapy are rare. The case reported is one of a man, 39 years old, with typical pernicious anemia. Liver and liver extract by mouth caused prompt improvement in the blood count. Neurologic symptoms occurred with the erythrocyte count at a normal level; and parenteral injections of liver extract were begun, intramuscularly. No untoward reactions occurred until after the fifteenth injection, when flushing of the face and neck occurred and lasted one hour. The following injection induced an urticarial eruption, and the following day there was generalized enlargement and tenderness of the lymph nodes. Recovery was complete in one week. Seventeen days after the last injection, another was given. This was followed immediately by clammy perspiration, an asthmatic paroxysm, rapid pulse rate, and bladder incontinence. Epinephrine induced relief. A new preparation of liver was secured and diluted. It was given in gradually increased doses without untoward reactions. Scratch tests were made with extracts of beef and pork with negative response. Extract of horse meat caused a raised area 14 mm. in diameter with a surrounding zone of erythema.

Serum Carditis: The Morphologic Cardiac Alterations in Man, Associated With Serum Disease. Clark, E.: J. A. M. A. **110:** 1008, 1938.

In a recent report by Clark and Kaplan, two instances were described in which patients received large doses of antipneumococcus serum, and came to autopsy four and nineteen days, respectively, following the signs of serum disease. The structural alterations in these cases consisted of proliferation of histiocytes in the mural and valvular endocardium and in the intima of the aorta, pulmonary, and coronary arteries, accompanied by multiplication of the interstitial cells of the myocardium and other viscera. Necrotizing arteritis and periarteritis of the smaller coronary arteries was a prominent feature in one case. It was felt that the composite picture was unlike that of any disease heretofore described. The coexistence of these lesions with clinical evidence of serum diseases and their similarity to those found in animals with protracted anaphylaxis induced by foreign sera, led the authors to suggest that these alterations were of hypergic nature and related to the administration of foreign serum.

Another case is here reported. A youth, aged 20 years, with acute anterior poliomyelitis affecting all limbs and the muscles of respiration died twelve days after the onset of the disease. Seven days before death, 40 c.c. of concentrated horse serum were given. Two days prior to death signs of serum disease appeared. Post-mortem study disclosed, in addition to the microscopic appearance of acute anterior poliomyelitis, diffuse infiltration of histiocytes in the subendocardial and connective tissues of the heart, accompanied by focal infiltration between the muscle fibers.

Musculo-Spiral Paralysis After Serum Injection. Brahdy, L.: Ann. Int. Med. **2**: 1911, 1938.

Peripheral nerve lesions after serum injection are not unknown, and a few years ago the literature on the subject was brought up to date. The present case concerns a man of 25 years, an alcoholic, who was given 1,500 units of tetanus antitoxin because of a laceration of the foot. One week later he complained of severe pain in the arms. These were tender and in some degree of spasm. A diagnosis of polyneuritis was made. Some weeks later a left wrist drop occurred which persisted several months.

Fourteen months after the first serum injection, there was occasion to again give 1,500 units of tetanus antitoxin, together with a prophylactic dose of "gas gangrene bacillus serum" (amount not stated). The injection was given subcutaneously. Eight hours later pain was felt in the left arm, and two days after that wrist drop occurred. There were no signs typical of serum sickness. The wrist drop recovered only partially three months later.

The Action of Zinc on Adrenalin Given Subcutaneously. Kohn, E., and Bulger, H. A.: Proc. Soc. Exper. Biol. & Med. **27**: 421, 1937.

Various quantities of zinc sulfate and adrenalin chloride were mixed and injected subcutaneously and the effects on blood pressure readings and blood sugar determinations made before and after injection were noted. It was found that zinc in small quantities diminishes the activity of adrenalin. It is believed that the retarded absorption of adrenalin permits much of it to be oxidized with consequent diminution of its effect.

Agranulocytosis and Sensitization to Pyramidon. Disselmeyer, H., and Zorn, O.: München. med. Wchnschr. **58**: 247, 1937.

A 48-year-old woman with arthritis of six weeks' standing, given 7 tablets of 0.3 gm. pyramidon each, developed fever, angina, and a leucopenia of 2,100 cells, of which 98 per cent were lymphocytes and monocytes. Some time after recovery an injection of 1 per cent pyramidon solution gave an immediate leucopenia, which seemed highly suggestive that the phenomenon is an allergic reaction.

Meningeal Reactions From Serum Therapy. Rocchi, F.: Policlinico **45**: 187, 1938.

Patients with poliomyelitis, tetanus, and meningococcus meningitis, who were given intraspinous serum therapy occasionally, developed reactions which were attributed to the heterologous serum. The reaction occurred within twenty-four hours after serum injection, reached its height in two days, and disappeared in about one week. No recurrence was observed on further intraspinal injections of serum. The reaction was characterized by an increase in meningeal symptoms, a polynuclear white cell response, and a colloidal gold curve resembling infectious meningitis. General serum sickness occasionally occurred. The author separates two possible causes of the phenomenon, one a toxic factor from the serum, the other an anaphylactoid reaction. These serum reactions can be identified by meningitic symptoms with sterile spinal fluid containing large numbers of polynuclear cells in diseases without meningeal involvement, and an increase of meningeal symptoms when meningitis is already present.